09-20-00

UTILITY PATENT APPLICATION TRANSMITTAL

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ADDRESS TO:

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

Attorney Docket No. 94,784-L

First Named Inventor Stojiljkovic

Express Mail No. EL028732014US

Total Pages 143

APPLICATION ELEMENTS	ACCOMPANYING APPLICATION PARTS
1. ☒ Transmittal Form with Fee	8. ☒ Assignment Papers
2. ☒ Specification (including claims and abstract) [Total Pages 78]	9. ☐ Power of Attorney
3. ☒ Drawings [Total Sheets 47]	10. ☐ English Translation Document (if applicable)
4. ☒ Oath or Declaration [Total Pages 4]	11. ☐ Information Disclosure Statement (IDS)
a. ☐ Newly executed	☐ PTO-1449 Form
b. ☒ Copy from prior application **[Note Boxes 5 and 17 below]**	☐ Copies of IDS Citations
i. ☐ Deletion of Inventor(s) Signed statement attached deleting inventor(s) named in the prior application	12. ☒ Preliminary Amendment
5. ☒ Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.	13. ☒ Return Receipt Postcard (Should be specifically itemized)
6. ☐ Microfiche Computer Program	14. ☒ Small Entity Statement(s)
7. ☒ Nucleotide and/or Amino Acid Sequence Submission	☐ Enclosed
a. ☐ Computer Readable Copy	☒ Statement filed in prior application; status still proper and desired
b. ☒ Paper Copy	15. ☐ Certified Copy of Priority Document(s)
c. ☐ Statement verifying above copies	16. ☐ Other:

17. ☒ This is a CONTINUING APPLICATION. Please note the following:

a. ☒ This is a ☒ Continuation ☐ Divisional ☐ Continuation-in-part of prior application U.S. Serial No. 08/537,361, filed October 2, 1995, now U.S. Patent No. 6,121,037, issued September 19, 2000, which is a continuation-in-part of U.S. Serial No. 08/326,670, filed October 18, 1994, now U.S. Patent No. 5,698,438, issued December 16, 1997.

b. ☐ Cancel in this application original claims ____of the prior application before calculating the filing fee.

c. ☐ Amend the specification by inserting before the first line the sentence: This is a ☐ continuation ☐ divisional ☐ continuation-in-part of application Serial No.

d. ☒ The prior application is assigned of record to Oregon Health Sciences University.

UTILITY PATENT APPLICATION TRANSMITTAL | Attorney Docket No. 94,784-L

APPLICATION FEES

BASIC FEE				$ 690.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total Claims	6 -20=		x $18.00	$ 0.00
Independent Claims	4 - 3=		x $78.00	$ 78.00
☐ Multiple Dependent Claims(s) if applicable			+$270.00	$ 0.00
			Total of above calculations =	$ 768.00
			Reduction by 50% for filing by small entity =	$1/2 384.00
☐ Assignment fee if applicable			+ $40.00	$ 0.00
			TOTAL =	$ 384.00

18. ☐ Please charge my Deposit Account No. 13-2490 in the amount of $

19. ☒ A check in the amount of $ 384.00 is enclosed.

20. The Commissioner is hereby authorized to credit overpayments or charge any additional fees of the following types to Deposit Account No. 13-2490:
    a. ☒ Fees required under 37 CFR 1.16.
    b. ☒ Fees required under 37 CFR 1.17.
    c. ☐ Fees required under 37 CFR 1.18.

21. ☒ The Commissioner is hereby generally authorized under 37 CFR 1.136(a)(3) to treat any future reply in this or any related application filed pursuant to 37 CFR 1.53 requiring an extension of time as incorporating a request therefor, and the Commissioner is hereby specifically authorized to charge Deposit Account No. 13-2490 for any fee that may be due in connection with such a request for an extension of time.

22. CERTIFICATE OF MAILING

I hereby certify that I directed that the correspondence identified above be deposited with the United States Postal Service as "Express Mail Post Office to Addressee" under 37 CFR § 1.10 on the date indicated below and is addressed to the Asst. Commissioner for Patents, Box Patent Application, Washington, DC 20231.

23. USPTO CUSTOMER NUMBER

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020306

24. CORRESPONDENCE ADDRESS

Name	**McDonnell Boehnen Hulbert & Berghoff**
Address	300 South Wacker Drive
City, State, Zip	Chicago, IL 60606

25. SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Name Reg. No.	Kevin E. Noonan, Reg. No.35,303
Signature	
Date	September 19, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 94,784-L)

PATENT

In application of:		)	
		)	
	Stojiljkovic *et al.*	)	**Before the Examiner:**
		)	
Serial No.	**To be assigned**	)	
		)	**Group Art Unit: 1641**
Filed:	**September 19, 2000**	)	
		)	
For:	**Novel Bacterial Hemoglobin Receptor Genes and Uses**	)	

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Please enter the following amendments to the above-identified divisional application.

AMENDMENT

IN THE SPECIFICATION:

On page 1, line 1, please insert the following:

– This application is a divisional of U.S. Serial No. 08/537,361, filed October 2, 1995, now U.S. Patent No. 6,121,037, issued September 19, 2000, which is a continuation-in-part of U.S. Serial No. 08/326,670, filed October 18, 1994, now U.S. Patent 5,698,438, issued December 16, 1997. The disclosures of each of these prior applications are considered as being part of the disclosure of the application and are explicitly incorporated by reference herein. –

On page 6, line 8, please replace "Figure 2" with --Figures 2A-2H--.

On page 6, line 11, please replace "Figure 2" with --Figures 2A-2H--.

On page 6, line 12, please replace "Figure 2" with --Figures 2A-2H--.

On page 6, line 27, please replace "Figure 7" with --Figures 7A-7I--.

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On page 7, line 1, please replace "Figure 7" with --Figures 7A-7I--.

On page 7, line 2, please replace "Figure 7" with --Figures 7A-7I--.

On page 7, line 17, please replace "Figure 8" with --Figures 8A-8I--.

On page 7, line 20, please replace "Figure 8" with --Figures 8A-8I--.

On page 7, line 21, please replace "Figure 8" with --Figures 8A-8I--.

On page 8, line 8, please replace "Figure 9" with --Figures 9A-9I--.

On page 8, line 10, please replace "Figure 9" with --Figures 9A-9I--.

On page 8, line 12, please replace "Figure 9" with --Figures 9A-9I--.

On page 8, line 26, please replace "Figure 2" with --Figures 2A-2H--.

Please indent page 8, line 27.

On page 9, line 1, please replace "Figure 7" with --Figures 7A-7I--.

On page 9, line 5, please replace "Figure 8" with --Figures 8A-8I--.

On page 9, line 8, please replace "Figure 9" with --Figures 9A-9I--.

On page 9, line 24, please replace "Figure 2" with --Figures 2A-2H--.

On page 9, line 27, please replace "Figure 7" with --Figures 7A-7I--.

On page 10, line 1, please replace "Figure 8" with --Figures 8A-8I--.

On page 10, line 5, please replace "Figure 9" with --Figures 9A-9I--.

On page 11, line 9, please replace "at" with --that--.

On page 12, line 5, please replace "Figure 2" with --Figures 2A-2H--.

On page 12, line 8, please replace "Figure 7" with --Figures 7A-7I--.

On page 12, line 12, please replace "Figure 8" with --Figures 8A-8I--.

On page 12, line 16, please replace "Figure 9" with --Figures 9A-9I--.

On page 12, line 20, please delete "each".

On page 13, line 18, please replace "Figure 2" with --Figures 2A-2H--.

On page 13, line 26, please replace "Figure 7" with --Figures 7A-7I--.

On page 14, line 4, please replace "Figure 8" with --Figures 8A-8I--.

On page 14, line 12, please replace "Figure 9" with --Figures 9A-9I--.

On page 14, line 19, please delete the comma after "human".

On page 15, line 15, please replace "Figure 2" with --Figures 2A-2H--.

On page 15, line 23, please replace "Figure 7" with --Figures 7A-7I--.

On page 16, line 1, please replace "Figure 8" with --Figures 8A-8I--.

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On page 16, line 11, please replace "Figure 9" with --Figures 9A-9I--.

On page 16, line 18, please insert a comma after "agonists".

On page 16, line 22, please replace "known or unknown" with --recognized or unrecognized--.

On page 17, line 17, please replace "Figure 2" with --Figures 2A-2H--.

On page 17, line 24, please replace "Figure 4" with --Figures 4A-4C--.

On page 18, line 9, please replace "Figure 7" with --Figures 7A-7I--.

On page 18, line 12, please replace "Figure 8" with --Figures 8A-8I--.

On page 18, line 15, please replace "Figure 9" with --Figures 9A-9I--.

On page 18, line 26, please replace "Figure 11" with --Figures 11A-11D--.

On page 19, line 6, please delete the comma after "by".

On page 19, line 7, please replace "Figure 2" with --Figures 2A-2H--.

On page 19, line 7, please replace "7" with --Figures 7A-7I--.

On page 19, line 7, please replace "8" with --Figures 8A-8I--.

On page 19, line 7, please replace "9" with --Figures 9A-9I--.

On page 19, line 10, please replace "Figure 2" with --Figures 2A-2H--.

On page 19, line 10, please replace "7" with --Figures 7A-7I--.

On page 19, line 10, please replace "8" with --Figures 8A-8I--.

On page 19, line 10, please replace "9" with --Figures 9A-9I--.

On page 19, line 19, please replace "Figure 2" with --Figures 2A-2H--.

On page 19, line 19, please replace "7" with --Figures 7A-7I--.

On page 19, line 19, please replace "8" with --Figures 8A-8I--.

On page 19, line 20, please replace "9" with --Figures 9A-9I--.

On page 20, line 15, please insert --can be prepared-- after "protein".

On page 23, line 12, please replace "manitol" with --mannitol–.

On page 23, line 17, please delete the comma after "salts".

On page 24, line 12, please replace "attenutated" with --attenuated--.

On page 27, line 16, please replace "The" with --Preferred--.

On page 28, line 6, please delete "also".

On page 28, line 26, please replace "a pathogenic" with --pathogenic--.

On page 31, line 2, please replace "**hemoglobin**" with --**Hemoglobin**--.

On page 31, line 5, please replace "haemin" with --hemin--.

On page 31, line 5, please replace "haemoglobin" with --hemoglobin--.

On page 31, line 19, please replace "onselective" with --on selective--.

On page 31, line 21, please replace "haemin" with --hemin--.

On page 33, line 2, please replace "Chemicals" with --Chemical--.

On page 33, line 13, please replace "*Cla*Ifragment" with --*Cla*I fragment--.

On page 35, line 11, please replace "Figure 2" with --Figures 2A-2H--.

On page 35, line 19, please replace "Figure 2" with --Figures 2A-2H--.

On page 36, line 5, please replace "fur-type" with --Fur-type--.

On page 36, line 11, please replace "Figure 2" with --Figures 2A-2H--.

On page 36, line 18, please replace "Figure 2" with --Figures 2A-2H--.

On page 37, line 2, please replace "Figure 4" with --Figures 4A-4C--.

On page 37, line 15, please insert a comma after "Postle".

On page 38, line 12, please replace "Table 2" with --Table II--.

On page 38, line 13, please replace "Table 2" with --Table II--.

On page 39, line 16, please replace "our" with --the--.

On page 39, line 16, please replace "probe ," with --probe,--.

On page 42, line 11, please replace "Figures 7" with --Figures 7A-7I--.

On page 42, line 11, please replace "8" with --Figures 8A-8I--.

On page 42, line 11, please replace "9" with --Figures 9A-9I--.

On page 42, line 16, please replace "Figures 10" with --Figure 10--.

On page 42, line 16, please replace "11" with --Figures 11A-11D--.

On page 42, line 24, please replace "Figure 11" with --Figures 11A-11D--.

If the Examiner in charge of this application believes it to be helpful, she is invited to contact the undersigned attorney by telephone at (312) 913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff

Date: September 16, 1999

By: _____
Kevin E. Noonan, Ph.D.
Reg. No. 35,303

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NOVEL BACTERIAL HEMOGLOBIN RECEPTOR GENES AND USES

This application is a continuation-in-part of U.S. patent application Serial No. 08/326,670, filed October 18, 1994.

This invention was made with government support under National Institute of Health grants R01 AI32493 and R01 AI22933. The government has certain rights to this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to hemoglobin receptor genes and the proteins encoded therefrom of certain bacterial species, particularly species of *Neisseria* bacteria. More particularly, this invention relates to hemoglobin receptor genes, polypeptides and peptides useful for preparing vaccines and antibodies against *Neisseria*, and methods and means for producing such peptides and polypeptides *in vitro*. Also provided are diagnostic and therapeutic methods and reagents useful in detecting and treating *Neisseria* infection and methods for developing novel and effective anti-*Neisseria* agents.

2. Background of the Invention

The *Neisseriae* comprise a genus of bacteria that includes two gram-negative species of pyogenic cocci pathogenic for humans: *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *N. meningitidis* is a major cause of bacterial meningitis in humans, especially children. The disease characteristically proceeds from asymptomatic carriage of the bacterium in the nasopharynx to invasion of the bloodstream and cerebrospinal fluid in susceptible individuals.

Neisseria meningitidis is one of the leading causes of bacterial meningitis in children and healthy adults in the world. The severity of the disease is evidenced by the ability of meningococci to cause the death of previously healthy individuals in less than 24 hours. *N. meningitidis* has a polysaccharide capsule whose diversity of component antigenic polysaccharide molecules has resulted in the classification of ten different serogroups. Of these, group A strains are the classic epidemic strains; group B and C are generally endemic strains, but C occasionally causes an epidemic outbreak. All known group A strains have the same protein antigens on their

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outer membranes, while group B strains have a dozen serotypes or groupings based on the presence of principal outer membrane protein antigens (as opposed to polysaccharides).

Survival of a pathogen such as *N. meningitidis* in a host depends on its ability to overcome a battery of host defense mechanisms. One nonspecific host defense mechanism against microbial intruders is to limit the availability of iron in tissues (Weinberg, 1984, *Physiological. Rev.* 64: 65-102), because iron is a necessary nutrient for most microbial pathogens. The vast majority of iron in the human adult is located intracellularly in the form of hemoglobin (76%) or ferritin (23%). The remainder can be found extracellularly bound to host iron-binding proteins such as transferrin and lactoferrin (Otto *et al.*, 1992, *Crit. Rev. Microbiol.* 18: 217-233).

Pathogenic bacteria have adapted to this iron-limiting environment by developing highly specific and effective iron assimilation systems. A large number of these bacteria secrete siderophores, small, non-protein iron chelators which, due to their extremely high affinity for iron (III), scavenge trace amounts of iron(III) from the environment and shuttle the iron back to the bacterial cell (Baggs and Neilands, 1987, *Microbiol. Rev.* 51: 509-518; Braun and Hantke, 1991, *in* Winkelmann (ed.), *Handbook of Microbial Iron Chelates*, CRC Press: Boca Raton, Fla., pp. 107-138.).

Alternatively, some bacterial pathogens, like *Neisseriae* species (Archilbald and DeVoe, 1979, *FEMS Microbiol. Lett.* 6: 159-162; Mickelson *et al.*, 1982, *Infect. Immun.* 35: 915-920; Dyer *et al.*, 1987, *Infect. Immun.* 55: 2171-2175), *Haemophilus influenzae* (Coulton and Pang, 1983, *Curr. Microbiol.* 9: 93-98; Schryvers, 1988, *Mol. Microbiol.* 2: 467-472; Jarosik *et al.*, 1994, *Infect. Immun.* 62: 2470-2477), *Vibrio cholerae* (Stoebner and Payne, 1988, *Infect. Immun.* 56: 2891-2895; Henderson and Payne, 1994, *J. Bacteriol.* 176: 3269-3277), *Yersiniae* (Stojiljkovic and Hantke, 1992, *EMBO J.* 11: 4359-4367) and *Actinobacillus pleuropneumoniae* (Gerlach *et al.*, 1992, *Infect. Immun.* 60: 3253-3261) have evolved more sophisticated mechanisms to sequester iron from the host. These pathogens can directly bind host's iron-binding proteins such as lactoferrin, transferrin, and heme-containing compounds, and use them as sole sources of iron.

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The importance of iron in the virulence of *N. meningitidis* was demonstrated by *in vivo* studies using mice as the animal model system (Calver *et al.*, 1976, *Can. J. Microbiol.* 22: 832-838; Holbien *et al.*, 1981, *Infect. Immun.* 34: 120-125). Specific iron-regulated outer membrane receptors have been shown to be involved in the binding and the utilization of lactoferrin- and transferrin-iron in *Neisseriae* (Schryvers and Morris, 1988, *Infect. Immun.* 56: 1144-1149 and *Mol. Microbiol.* 2: 281-288; Legrain *et al.*, 1993, *Gene* 130: 81-90; Pettersson *et al.*, 1993, *Infect. Immun.* 61: 4724-4733 and 1994, *J. Bacteriol.* 176: 1764-1766). These receptors share significant amino acid similarity and, most probably, also the mechanism of iron internalization, with receptors for siderophores and vitamin B12 of other Gram-negative bacteria (Cornelissen *et al.*, 1993, *J. Bacteriol.* 174: 5788-5797). In contrast, the mechanism by which *Neisseriae* utilize hemoglobin- and hemin-iron as well as the components involved have so far not been described.

Recently, several proteins with hemoglobin-binding and/or hemin-binding activities have been identified in total membranes of iron-limited *N. meningitidis* and *N. gonorrhoeae*.

Lee and Hill, 1992, *J. gen. Microbiol.* 138: 2647-2656 disclose the specific hemoglobin binding by isolated outer membranes of *N. meningitidis*.

Martek and Lee, 1994, *Infect. Immun.* 62: 700-703 disclosed that acquisition of heme iron by *N. meningitidis* does not involve meningococcal transferrin-binding proteins.

Lee, 1994, *Microbiol.* 140: 1473-1480 describes the biochemical isolation and characterization of hemin binding proteins from *N. meningitidis*.

The precise role of these proteins in hemin and/or hemoglobin utilization remains unclear at present, although these proteins are likely to be components of a hemin-utilization system in *N. meningitidis*.

The dependence on host iron stores for *Neisseria* growth is a potentially useful route towards the development of novel and effective therapeutic intervention strategies. Historically, infections of both *N. meningitidis* and *N. gonorrhoeae* were treated chemoprophylactically with sulfonamide drugs. However, with the development of sulfonamide-resistant strains came the necessity of using alternative modes of therapy such as antibiotic treatment. More recently, the drug treatment of choice includes the administration of high grade penicillin. However, the

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success of antimicrobial treatment is decreased if therapy is not initiated early after infection.

Gonococcal infection has also been treated with penicillin, ampicillin, or amoxicillin, tetracycline hydrochloride, and spectinomycin. Unfortunately, because the incidence of infections due to penicillinase-producing bacteria has increased, several new, more expensive ß-lactam antibiotics have been used in treatment. Despite the fact that existing antibiotics have decreased the serious consequences of gonorrhea, their use has not lowered the incidence of the infection in the general population.

Prevention of meningococcal disease has been attempted by chemoprophylaxis and immunoprophylaxis. At present, rifampin and minocycline are used, but only for humans in close contact with an infected person as this treatment has a number of disadvantages. The only commercially available vaccine against meningococcal meningitis has as its major component the bacterial polysaccharide capsule. In adults this vaccine protects against serogroups A, C, Y and W135. It is not effective against serogroup B, and is ineffective in children against serogroup C. Thus far, immunoprophylatic preventive treatment has not been available for *N. gonorrhoeae*.

Thus, what is needed are better preventative therapies for meningococcal meningitis and gonorrhea including more effective, longer lasting vaccines which protect across all of the serogroups of *N. meningitidis* and all the serotypes of *N. gonorrhoeae*. In addition, better methods are need to treat meningococcal and gonococcal infection.

SUMMARY OF THE INVENTION

The present invention relates to the cloning, expression and functional characterization of genes encoding bacterial hemoglobin receptor proteins. Specifically, the invention relates to genes encoding hemoglobin receptor proteins from *Neisseria* species, in particular *Neisseria meningitidis* and *N. gonorrhoeae*. The invention comprises species of nucleic acids having a nucleotide sequence encoding novel bacterial hemoglobin receptor proteins. Also provided by this invention is the deduced amino acid sequence of the cognate hemoglobin receptor proteins of these bacterial genes.

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The invention provides nucleic acids, nucleic acid hybridization probes, recombinant expression constructs capable of expressing the hemoglobin receptor protein of the invention in cultures of transformed cells, preferably bacterial cells, and such cultures of transformed bacterial cells that express the hemoglobin receptor proteins of the invention. The invention also provides gene knockout vectors for inactivating the hemoglobin receptor protein gene in cells, particularly cells of *Neisseria* species, *via*, for example, homologous recombination and other mechanisms, and cultures of such hemoglobin receptor protein null mutant cells.

The invention also provides homogeneous preparations of the bacterial hemoglobin receptor proteins of the invention, as well as antibodies against and epitopes of the hemoglobin receptor protein. Methods for characterizing this receptor protein and methods for using the protein in the development of agents having pharmacological uses related to this receptor, particularly bactericidal and bacteriostatic uses, are also provided by the invention.

In other embodiments of this invention are provided diagnostic methods and reagents encompassing the use of the anti-*Neisseria* hemoglobin receptor protein antibodies of the invention. Still further embodiments provided herein include therapeutic methods and reagents encompassing the use of the anti-*Neisseria* hemoglobin receptor protein antibodies of the invention. Even more embodiments include diagnostic methods and reagents encompassing the use of the *Neisseria* hemoglobin receptor protein-encoding nucleic acids of the invention, as sensitive probes for the presence of *Neisseria* infection using nucleic acid hybridization techniques and/or *in vitro* amplification methodologies. Yet additional embodiments of the invention include therapeutic methods and reagents encompassing the use of the *Neisseria* hemoglobin receptor protein-encoding nucleic acids of the invention, comprising recombinant expression constructs engineered to produce antisense transcripts of the *Neisseria* hemoglobin receptor gene and fragments thereof, as well as recombinant knockout vectors of the invention. The invention also provides the *Neisseria* hemoglobin receptor protein and epitopes thereof as components of vaccines for the development of non-disease associated immunity to pathological infection with bacteria of *Neisseria* species.

In a first aspect, the invention provides a nucleic acid having a nucleotide sequence encoding a bacterial hemoglobin receptor protein gene. In a preferred embodiment, the bacterial

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hemoglobin receptor protein gene is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*, serotype C. In a particular example of this embodiment, the nucleic acid comprises a 3.3 kilobase (kb) *Bam*HI/*Hin*dIII fragment of *N. meningitidis* genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of 2376 nucleotides of *N. meningitidis* genomic DNA encoding 792 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis* hemoglobin receptor gene is the sequence depicted in Figure 2 (SEQ ID No:1). It will be understood that the *N. meningitidis* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in Figure 2 (SEQ. ID No.:2). Thus, it will be understood that the particular nucleotide sequence depicted in Figure 2 (SEQ. ID. No.:1) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. meningitidis* hemoglobin receptor protein disclosed herein.

In another particularly preferred embodiment of this aspect of the invention, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*, serotype A. In a particular example of this embodiment, the nucleic acid comprises a 2373 basepair (bp) polymerase chain reaction-amplified fragment of *N. meningitidis*, serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of 2373 nucleotides of *N. meningitidis* genomic DNA encoding 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis* hemoglobin receptor gene is the sequence depicted in Figure 7 (SEQ ID No:3). It will be understood that the *N. meningitidis* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence

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being represented in Figure 7 (SEQ. ID No.:4). Thus, it will be understood that the particular nucleotide sequence depicted in Figure 7 (SEQ. ID. No.:3) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. meningitidis* hemoglobin receptor protein disclosed herein.

In another particularly preferred embodiment of this aspect of the invention, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*, serotype B. In a particular example of this embodiment, the nucleic acid comprises a 2376 basepair (bp) polymerase chain reaction-amplified fragment of *N. meningitidis*, serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of 2373 nucleotides of *N. meningitidis* genomic DNA encoding 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis* hemoglobin receptor gene is the sequence depicted in Figure 8 (SEQ ID No:5). It will be understood that the *N. meningitidis* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in Figure 8 (SEQ. ID No.:6). Thus, it will be understood that the particular nucleotide sequence depicted in Figure 8 (SEQ. ID. No.:5) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. meningitidis* hemoglobin receptor protein disclosed herein.

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In yet other preferred embodiments, the invention provides nucleic acid encoding a hemoglobin receptor protein gene isolated from *Neisseria gonorrhoeae*. In a particular example of this embodiment, the nucleic acid comprises a 2378 basepair (bp) polymerase chain reaction-amplified fragment of *N. gonorrhoeae* genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of 2373 nucleotides of *N. gonorrhoeae* genomic DNA encoding 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. gonorrhoeae* hemoglobin receptor gene is the sequence depicted in Figure 9 (SEQ ID No:7). It will be understood that the *N. gonorrhoeae* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in Figure 9 (SEQ. ID No.:8). Thus, it will be understood that the particular nucleotide sequence depicted in Figure 9 (SEQ. ID. No.:7) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. gonorrhoeae* hemoglobin receptor protein disclosed herein.

The invention also provides bacterial hemoglobin receptor proteins. In a preferred embodiment, the bacterial hemoglobin receptor protein is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein is isolated from *Neisseria meningitidis*. In a particular example of this embodiment, the protein is derived from *N. meningitidis*, serotype C and comprises an amino acid sequence of 792 amino acids. In this embodiment of the invention, the amino acid sequence of the *N. meningitidis*, serotype C hemoglobin receptor protein is the sequence depicted in Figure 2 (SEQ ID No:2).
In another example of this embodiment, the protein is derived from *N. meningitidis*, serotype A and comprises an amino acid sequence of 790 amino acids. In this embodiment of the invention, the amino acid sequence of the *N. meningitidis*, serotype A hemoglobin receptor

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protein is the sequence depicted in Figure 7 (SEQ ID No:4). In yet another example of this embodiment, the protein is derived from *N. meningitidis*, serotype B and comprises an amino acid sequence of 791 amino acids. In this embodiment of the invention, the amino acid sequence of the *N. meningitidis*, serotype B hemoglobin receptor protein is the sequence depicted in Figure 8 (SEQ ID No:6). The invention also provides hemoglobin receptor protein derived from *N. gonorrhoeae*. In this embodiment of the invention, the protein comprises an amino acid sequence of 791 amino acids, and the amino acid sequence of the *N. gonorrhoeae* hemoglobin receptor protein is the sequence depicted in Figure 9 (SEQ ID No:8). Also explicitly encompassed within the scope of this invention are related bacterial hemoglobin receptor proteins, particularly such proteins isolated from *Neisseria* species, having essentially the same amino acid sequence and substantially the same biological properties as the hemoglobin receptor protein encoded by the *N. meningitidis* and *N. gonorrhoeae* nucleotide sequences described herein.

In another aspect, the invention provides a homogeneous preparation of an approximately 85.5 kiloDalton (kD) bacterial hemoglobin receptor protein or derivative thereof, said size being understood to be the size of the protein before any post-translational modifications thereof. Also provided is a 90kD embodiment of the receptor as determined by sodium dodecyl sulfate/ polyacrylamide gel electrophoresis under reducing conditions. In a preferred embodiment, the bacterial hemoglobin receptor protein is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein is isolated from *Neisseria meningitidis*. In one embodiment of this aspect of the invention, the protein is isolated from *N. meningitidis*, serotype C and the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence of the hemoglobin receptor protein shown in Figure 2 (SEQ ID No:2). In a second embodiment of this aspect of the invention, the protein is isolated from *N. meningitidis*, serotype A and the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence of the hemoglobin receptor protein shown in Figure 7 (SEQ ID No:4). In a third embodiment of this aspect of the invention, the protein is isolated from *N. meningitidis*, serotype B and the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof

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preferably is the amino acid sequence of the hemoglobin receptor protein shown in Figure 8 (SEQ ID No:6). The invention also provides a homogeneous preparation of a bacterial hemoglobin receptor protein isolated from *N. gonorrhoeae*. In a preferred embodiment, the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence of the hemoglobin receptor protein shown in Figure 9 (SEQ ID No:8).

This invention provides nucleotide probes derived from the nucleotide sequences herein provided. The invention includes probes isolated from either complementary DNA (cDNA) copies of bacterial messenger RNA (mRNA) or bacterial genomic DNA (gDNA), as well as probes made synthetically or by *in vitro* amplification methods using the sequence information provided herein. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or *in vitro* amplified probes made using cDNA or genomic clones embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the nucleotide sequence information of cDNA or genomic clone embodiments of the invention.

It is a further object of this invention to provide such nucleic acid hybridization probes to detect the presence of bacteria of *Neisseria* species, particularly *N. meningitidis* and *N. gonorrhoeae*, in a biological sample in the diagnosis of a *Neisseria* infection in a human. Such a biological sample preferably includes blood, urine, semen, mucus, cerebrospinal fluid, peritoneal fluid and ascites fluids, as well as cell scrapings from the epithelium of the mouth, urethra, anus and rectum, and other organs.

The present invention also includes peptides encoded by the nucleotide sequences comprising the nucleic acid embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of hemoglobin receptor protein-specific antibodies. The invention also comprises such antibodies, preferably monoclonal antibodies, and cells and cultures of cells producing such antibodies.

Thus, the invention also provides antibodies against and epitopes of bacterial hemoglobin receptor proteins of the invention. It is an object of the present invention to provide antibodies that are immunologically reactive to the bacterial hemoglobin receptor proteins of the invention.

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It is a particular object to provide monoclonal antibodies against these bacterial hemoglobin receptor proteins. In a preferred embodiment, antibodies provided are raised against bacterial hemoglobin receptor protein isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria meningitidis* serotypes A, B or C. In additional particularly preferred embodiment, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria gonorrhoeae*.

Hybridoma cell lines producing such antibodies are also objects of the invention. It is envisioned at such hybridoma cell lines may be produced as the result of fusion between a non-immunoglobulin producing mouse myeloma cell line and spleen cells derived from a mouse immunized with purified hemoglobin receptor protein or a cell expressing antigens or epitopes of bacterial hemoglobin receptor proteins of the invention. The present invention also provides hybridoma cell lines that produce such antibodies, and can be injected into a living mouse to provide an ascites fluid from the mouse that is comprised of such antibodies. In a preferred embodiment, antibodies provided are raised against bacterial hemoglobin receptor protein isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria meningitidis*, serotypes A, B or C. In additional particularly preferred embodiment, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria gonorrhoeae*.

It is a further object of the invention to provide immunologically-active epitopes of the bacterial hemoglobin receptor proteins of the invention. Chimeric antibodies immunologically reactive against the bacterial hemoglobin receptor proteins of the invention are also within the scope of this invention. In a preferred embodiment, antibodies and epitopes provided are raised against or derived from bacterial hemoglobin receptor protein isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, such antibodies and epitopes are specific for the hemoglobin receptor protein isolated from *Neisseria meningitidis*, serotypes A, B or C. In additional particularly preferred embodiment, such antibodies and epitopes are specific for the hemoglobin receptor protein isolated from *Neisseria gonorrhoeae*.

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The present invention provides recombinant expression constructs comprising a nucleic acid encoding a bacterial hemoglobin receptor protein wherein the construct is capable of expressing the encoded hemoglobin receptor protein in cultures of cells transformed with the construct. Preferred embodiments of such constructs comprise the *N. meningitidis*, serotype C hemoglobin receptor gene depicted in Figure 2 (SEQ ID No.:1), such constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct. Additional preferred embodiments of such constructs comprise the *N. meningitidis*, serotype A hemoglobin receptor gene depicted in Figure 7 (SEQ ID No.:3), such constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct. Further additional preferred embodiments of such constructs comprise the *N. meningitidis*, serotype B hemoglobin receptor gene depicted in Figure 8 (SEQ ID No.:5), such constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct. The invention also provides recombinant expression constructs encoding a hemoglobin receptor protein gene isolted from *ZN. gonorrhoeae*. In a particularly preferred embodiment, such constructs comprise the *N. gonorrhoeae* hemoglobin receptor gene depicted in Figure 9 (SEQ ID No.:7), the constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct.

The invention also provides cultures of cells, preferably bacterial cells, having been transformed with the recombinant expression constructs of the invention, each such cultures being capable of and in fact expressing the bacterial hemoglobin receptor protein encoded in the transforming construct.

The present invention also includes within its scope protein preparations of prokaryotic cell membranes containing the bacterial hemoglobin receptor protein of the invention, derived from cultures of prokaryotic cells transformed with the recombinant expression constructs of the invention.

The invention also provides diagnostic reagents and methods for using such reagents for detecting the existence of an infection in a human, with bacteria of a *Neisseria* species. In preferred embodiments, such diagnostic reagents comprise antibodies that are immunologically

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reactive with a bacterial hemoglobin receptor protein. In a preferred embodiment, such antibodies are raised against a bacterial hemoglobin receptor protein isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria meningitidis*, serotypes A, B or C. In additional particularly preferred embodiments, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria gonorrhoeae*.

In yet another embodiment of this aspect of the invention are provided diagnostic reagents and methods for using such reagents wherein said reagents are nucleic acid hybridization probes comprising a bacterial hemoglobin receptor gene. In a preferred embodiment, the bacterial hemoglobin receptor protein gene is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*. In particular examples of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 3.3 kilobase (kb) *Bam*HI/*Hin*dIII fragment of *N. meningitidis*, serotype C genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2376 nucleotides of *N. meningitidis*, serotype C genomic DNA encoding 792 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype C hemoglobin receptor gene is the sequence depicted in Figure 2 (SEQ ID No:1). In another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 2373bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2370 nucleotides of *N. meningitidis*, serotype A genomic DNA encoding 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype A hemoglobin receptor gene is the sequence depicted in Figure 7 (SEQ ID No:3). In yet another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 2376bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, serotype B genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment

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of an open reading frame of 2373 nucleotides of *N. meningitidis*, serotype B genomic DNA encoding 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype B hemoglobin receptor gene is the sequence depicted in Figure 8 (SEQ ID No:5). The invention also provides nucleic acid hybridization probes comprising a bacterial hemoglobin receptor gene isolated from *N. gonorrhoeae*. In a preferred embodiment of this aspect of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 2378bp, polymerase chain reaction-amplified fragment of *N. gonorrhoeae* genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2373 nucleotides of *N. gonorrhoeae* genomic DNA encoding 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. gonorrhoeae* hemoglobin receptor gene is the sequence depicted in Figure 9 (SEQ ID No:7). It will be understood that the term "specifically-hybridizing" when used to describe a fragment of a nucleic acid encoding a bacterial hemoglobin receptor gene is intended to mean that nucleic acid hybridization of such a fragment is stable under high stringency conditions of hybridization and washing as the term "high stringency" would be understood by those having skill in the molecular biological arts.

Also provided by the invention are therapeutic agents and methods for using such agents for treating the an infection in a human, with bacteria of a *Neisseria* species. In preferred embodiments, such agents comprise antibodies that are immunologically reactive with a bacterial hemoglobin receptor protein. In a preferred embodiment, such antibodies are raised against a bacterial hemoglobin receptor protein isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria meningitidis*, serotypes A, B or C. In additional preferred embodiments, such antibodies are specific for the hemoglobin receptor protein isolated from *Neisseria gonorrhoeae*. Therapeutic agents provided in this aspect of the invention comprise such antibodies in a pharmaceutically-acceptable carrier, along with appropriate adjuvants and the like. In additional embodiments, such antibodies are covalently conjugated to a bactericidal

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or bacteriostatic agent effective against bacteria of *Neisseria* species, preferably *N. meningitidis* and *N. gonorrhoeae*.

In yet another embodiment of this aspect of the invention are provided therapeutic reagents and methods for using such reagents wherein said reagents comprise recombinant expression constructs of the invention, or a homologue thereof that expresses the nucleic acid encoding a hemoglobin receptor in an antisense orientation. In a preferred embodiment, the bacterial hemoglobin receptor protein gene is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*. In particular examples of this embodiment of the invention, the nucleic acids comprise a specifically-hybridizing fragment of a 3.3 kilobase (kb) *Bam*HI/*Hin*dIII fragment of *N. meningitidis*, serotype C genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2376 nucleotides of *N. meningitidis*, serotype C genomic DNA encoding 792 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype C hemoglobin receptor gene is the sequence depicted in Figure 2 (SEQ ID No:1). In another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 2373bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2370 nucleotides of *N. meningitidis*, serotype A genomic DNA encoding 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype A hemoglobin receptor gene is the sequence depicted in Figure 7 (SEQ ID No:3). In yet another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 2376bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, serotype B genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2373 nucleotides of *N. meningitidis*, serotype B genomic DNA encoding 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype B hemoglobin receptor gene

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is the sequence depicted in Figure 8 (SEQ ID No:5). The invention also provides recombinant expression constructs of the invention, or a homologue thereof that expresses the nucleic acid encoding a hemoglobin receptor in an antisense orientation, wherein the nucleic acid encodes a bacterial hemoglobin receptor gene isolated from *N. gonorrhoeae*. In a preferred embodiment of this aspect of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 2378bp, polymerase chain reaction-amplified fragment of *N. gonorrhoeae* genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2373 nucleotides of *N. gonorrhoeae* genomic DNA encoding 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. gonorrhoeae* hemoglobin receptor gene is the sequence depicted in Figure 9 (SEQ ID No:7).

The invention also provides a method for screening compounds for their ability to inhibit, facilitate or modulate the biochemical activity of a bacterial hemoglobin receptor protein of the invention, for use in the *in vitro* screening of novel agonist and antagonist compounds and novel bactericidal and bacteriostatic agents specific for the hemoglobin receptor protein. In preferred embodiments, cells transformed with a recombinant expression construct of the invention are contacted with such a compound, and the binding capacity of the compounds, as well as the effect of the compound on binding of other, known hemoglobin receptor agonists such as hemoglobin and hemin, and antagonists, is assayed. Additional preferred embodiments comprise quantitative analyses of such effects.

The present invention is also useful for the detection of bactericidal and/or bacteriostatic analogues, agonists or antagonists, known or unknown, of a bacterial hemoglobin receptor protein, preferably derived from bacteria of *Neisseria* species, most preferably isolated from *N. meningitidis,* wherein such compounds are either naturally occurring or embodied as a drug.

The invention also provides vaccines for immunizing a human against infection with pathogenic bacteria of *Neisseria* species, the vaccines comprising the hemoglobin binding proteins of the invention or antigenic fragments thereof. In a preferred embodiment, the vaccines of the invention comprise cells expressing a hemoglobin receptor binding protein of the invention, or an antigenic fragment thereof, preferably wherein said cells are attenuated varieties

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of cells adapted for growth in humans, *i.e.*, wherein such cells are non-pathogenic and do not cause bactermia, endotoxemia or sepsis. Examples of such attenuated varieties of cells include attenuated strains of *Salmonella* species, for example *Salmonella typhi* and *Salmonella typhimurium*, as well as other attenuated bacterial species. Also provided by the invention are recombinant expression constructs as disclosed herein useful *per se* as vaccines, for introduction into an animal and production of an immunologic response to bacterial hemoglobin receptor protein antigens encoded therein.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

Figure 1 is a schematic drawing of the restriction enzyme digestion map of a *N. meningitidis* cosmid clone and subclones thereof derived as described in Example 2.

Figure 2 illustrates the nucleotide (SEQ ID No.:1) and deduced amino acid (SEQ ID No.:2) sequences of the *N. meningitidis* hemoglobin receptor protein encoded in a 3.3 kb *Bam*HI/*Hin*dIII DNA fragment.

Figure 3 presents a photograph of a stained SDS/ 10% PAGE electrophoresis gel showing the results of *in vitro* expression of the *N. meningitidis* hemoglobin receptor gene product as an approximately 90 kilodalton protein, and β-lactamase protein having a molecular weight of about 30.0 kilodaltons used as a molecular weight marker.

Figure 4 presents an amino acid sequence comparison between portions of the *N. meningitidis* transferrin receptor Tbp1 (SEQ ID No.:9), the *N. meningitidis* lactoferrin receptor LbpA (SEQ ID No.:10), and *N. meningitidis* hemoglobin receptor HmbR (SEQ ID No.:2).

Figure 5 illustrates Southern hybridization analysis of chromosomal DNA from *N. meningitidis* 8013 and the MC8013*hmbR* mutant using a *Bam*HI-*Sal*I fragment of the *hmb* gene as probe labeled using a DIG nonradioactive DNA labelling and detection kit (Boehringer

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Mannheim Biochemicals, Indianapolis, IN). Lane 1 contains DNA from *N. meningitidis* strain MC8013, digested with *Cla*I; lane 2 is MC8031*hmbR* DNA digested with *Cla*I; lane 3, is MC8013 DNA digested with *Bam*HI and *Sal*I; and lane 4 is MC8013*hmbR* DNA digested with *Bam*HI and *Sal*I.

Figure 6 is a graph describing the course of infection using *N. meningitidis* wild type (MC8013) and *hmbR* mutant strains in an *in vivo* rat infant infection model. Each strain was injected intraperitoneally (2 x 10^6 CFU) into three infant inbred Lewis rats. The results represent the average of two similarly-performed experiments.

Figure 7 illustrates the nucleotide (SEQ ID No.:3) and deduced amino acid (SEQ ID No.:4) sequences of the *N. meningitidis*, serotype A hemoglobin receptor protein encoded on a 2373bp polymerase chain reaction-amplified DNA fragment.

Figure 8 illustrates the nucleotide (SEQ ID No.:5) and deduced amino acid (SEQ ID No.:6) sequences of the *N. meningitidis*, serotype B hemoglobin receptor protein encoded on a 2376bp polymerase chain reaction-amplified DNA fragment.

Figure 9 illustrates the nucleotide (SEQ ID No.:7) and deduced amino acid (SEQ ID No.:8) sequences of the *N. gonorrhoeae* hemoglobin receptor protein encoded on a 2376bp polymerase chain reaction-amplified DNA fragment.

Figure 10 represents a schematic of a nucleic acid sequence comparison between the hemoglobin receptor proteins derived from *N. meningitidis*, serotypes A (SEQ ID No.:3), B (SEQ ID No.:5) and C (SEQ ID No.:1) and from *N. gonorrhoeae* (SEQ ID No.:7), wherein the direction of trascription of the genes is in the direction of the arrow, and the following abbreviations refer to restriction endonuclease sites: H represents *Hin*dIII; N represents *Not*I; Bg represents *Bgl*I; Bs represents *Bss*HI; Nr represents *Nru*I; Cl represents *Cla*I; P represents *Pst*I; Sa represents *Sac*I; Av represents *Ava*I; B represents *Bam*HI; S represents *Sal*I; EV represents *Eco*RV; Sh represents *Sph*I; and Sy represents *Sty*I.

Figure 11 presents an amino acid sequence comparison between the hemoglobin receptor proteins derived from *N. meningitidis*, serotypes A (SEQ ID No.:4), B (SEQ ID No.:6) and C (SEQ ID No.:2) and from *N. gonorrhoeae* (SEQ ID No.:8).

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "bacterial hemoglobin receptor" as used herein refers to bacterial proteins comprising the outer membrane of Gram negative bacteria, which specifically mediate transit of hemoglobin-derived hemin, as well as hemin from other sources, through the outer membrane of such bacteria and into the periplasmic space. The bacterial hemoglobin receptor proteins of the invention are characterized by, first, an amino acid sequence that is essentially the sequence depicted in Figures 2 (SEQ ID No.:2), 7 (SEQ ID No.:4), 8 (SEQ ID No.:6) and 9 (SEQ ID No.:8). The bacterial hemoglobin receptor proteins of the invention are further characterized by having substantially the same biological activity as a protein having the amino acid sequence depicted in Figures 2 (SEQ ID No.:2), 7 (SEQ ID No.:4), 8 (SEQ ID No.:6) and 9 (SEQ ID No.:8). This definition is intended to encompass naturally-occurring variants and mutant proteins, as well as genetically engineered variants made by man.

Cloned, isolated and purified nucleic acid provided by the present invention may encode a bacterial hemoglobin receptor protein of any *Neisseria* species of origin, including, most preferably, *Neisseria meningitidis* species and serotypes thereof and *Neisseria gonorhoeae* species.

The nucleic acid hybridization probes provided by the invention comprise DNA or RNA having all or a specifically-hybridizing fragment of the nucleotide sequence of the hemoglobin receptor protein as depicted in Figures 2 (SEQ ID No.:1), 7 (SEQ ID No.:3), 8 (SEQ ID No.:5) and 9 (SEQ ID No.:7), or any portion thereof effective in nucleic acid hybridization. Mixtures of such nucleic acid hybridization probes are also within the scope of this embodiment of the invention. Nucleic acid probes as provided herein are useful for detecting the presence of a bacteria, *inter alia*, in a human as the result of an infection, in contaminated biological samples and specimens, in foodstuffs and water supplies, or in any substance that may come in to contact with the human. Specific hybridization will be understood to mean that the nucleic acid probes of the invention are capable of forming stable, specific hybridization to bacterially-derived DNA or RNA under conditions of high stringency, as the term "high stringency" would be understood by those with skill in the art (*see, for example*, Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Hames and Higgins, eds., 1985, Nucleic Acid Hybridization, IRL Press, Oxford, U.K.).

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Hybridization will be understood to be accomplished using well-established techniques, including but not limited to Southern blot hybridization, Northern blot hybridization, *in situ* hybridization and Southern hybridization to polymerase chain reaction product DNAs. The invention will thus be understood to provide oligonucleotides, specifically, pairs of oligonucleotides, for use as primers in support of *in vitro* amplification of bacterial hemoglobin receptor genes and mRNA transcripts.

The production of proteins such as bacterial hemoglobin receptor proteins from cloned genes by genetic engineering means is well known in this art. The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art. It will be understood from the following discussion that the hemoglobin receptor protein genes of this invention are particularly advantageous, since expression of such proteins by bacteria, including non-*Neisseria* species of bacteria, can complement certain auxotrophic mutants of said transformed bacteria otherwise unable to subsist absent supplementation of the growth media with iron (III).

DNA encoding a bacterial hemoglobin receptor protein, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the nucleic acid sequence information from the bacterial hemoglobin receptor protein disclosed herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with know procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, bacterial hemoglobin receptor protein-encoding nucleic acids may be obtained by use of the polymerase chain reaction (PCR) procedure, using appropriate pairs of PCR oligonucleotide primers corresponding to nucleic acid sequence information derived from a bacterial hemoglobin receptor protein as provided herein. *See* U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis, as specifically disclosed herein in Example 9 below. In another alternative, such bacterial hemoglobin receptor protein-encoding

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nucleic acids may be isolated from auxotrophic cells transformed with a bacterial hemoglobin receptor protein gene, thereby relieved of the nutritional requirement for uncomplexed iron (III).

Any bacterial hemoglobin receptor protein of the invention may be synthesized in host cells transformed with a recombinant expression construct comprising a nucleic acid encoding the bacterial hemoglobin receptor protein. Such recombinant expression constructs can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding a bacterial hemoglobin receptor protein and/or to express DNA encoding a bacterial hemoglobin receptor protein. For the purposes of this invention, a recombinant expression construct is a replicable DNA construct in which a nucleic acid encoding a bacterial hemoglobin receptor protein is operably linked to suitable control sequences capable of effecting the expression of the bacterial hemoglobin receptor protein in a suitable host cell.

The need for such control sequences will vary depending upon the host cell selected and the transformation method chosen. Generally, bacterial control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites (the Shine-Delgarno sequence), and sequences which control the termination of transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. *See*, Sambrook *et al.*, 1989, *ibid.*

Vectors useful for practicing the present invention include plasmids and virus-derived constructs, including phage and particularly bacteriophage, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. A preferred vector is pLAFR2 (*see* Riboli *et al.*, 1991, *Microb. Pathogen.* 10: 393-403).

Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising nucleic acid encoding a bacterial hemoglobin receptor protein. Preferred host cells are cells of *Neisseria* species, particularly *N. meningitidis*, as well as *Salmonella typhi* and *Salmonella*

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typhimurium species, and *Escherichia coli* auxotrophic mutant cells (*hemA aroB*). Transformed host cells may express the bacterial hemoglobin receptor protein, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridization probe DNA need not express the receptor protein. When expressed, the bacterial hemoglobin receptor protein of the invention will typically be located in the host cell outer membrane. *See*, Sambrook *et al.*, *ibid.*

Cultures of bacterial cells, particularly cells of *Neisseria* species, and certain *E. coli* mutants, are a desirable host for recombinant bacterial hemoglobin receptor protein synthesis. In principal, any bacterial cell auxotrophic for uncomplexed iron (III) is useful for selectively growing bacterial hemoglobin receptor protein-transformed cells. However, for this purpose, well-characterized auxotrophs, such as *E. coli hemA aroB* mutants are preferred.

The invention provides homogeneous compositions of a bacterial hemoglobin receptor protein produced by transformed cells as provided herein. Each such homogeneous composition is intended to be comprised of a bacterial hemoglobin receptor protein that comprises at least 90% of the protein in such a homogenous composition. The invention also provides membrane preparations from cells expressing a bacterial hemoglobin receptor protein as the result of transformation with a recombinant expression construct of the invention, as described herein.

Bacterial hemoglobin receptor proteins, peptide fragments thereof and membranes derived from cells expressing such proteins in accordance with the present invention may be used for the production of vaccines effective against bacterial infections in a human, with pathogenic microorganisms expressing such bacterial hemoglobin receptor proteins. Such vaccines preferably would be effective in raising an immunological response against bacteria of *Neisseria* species, most preferably *N. meningitidis* and *N. gonorhoeae*. Also encompassed within the vaccines provided by the invention are recombinant expression constructs as disclosed herein useful *per se* as vaccines, for introduction into an animal and production of an immunologic response to bacterial hemoglobin receptor protein antigens encoded therein.

Preparation of vaccines which contain polypeptide or polynucleotide sequences as active ingredients is well understood in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions. However, solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be

emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1 to 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of manitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25 to 70%.

The polypeptides of the invention may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts, include the acid additional salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

In another embodiment, such vaccines are provided wherein the bacterial hemoglobin receptor proteins or peptide fragments thereof are present in the intact cell membranes of cells expressing such proteins in accordance with the present invention. In preferred embodiments, cells useful in these embodiments include attenuated varieties of cells adapted to growth in humans. Most preferably, said cells are attenuated varieties of cells adapted for growth in humans, *i.e.*, wherein such cells do not cause frank disease or other pathological conditions, such as bactermia, endotoxemia or sepsis. For the purposes of this invention, "attenuated" cells

will be understood to encompass prokaryotic and eukaryotic cells that do not cause infection, disease, septicemia, endotoxic shock, pyrogenic shock, or other serious and adverse reactions to administration of vaccines to an animal, most preferably a human, when such cells are introduced into the animal, whether such cells are viable, living, heat-, chemically- or genetically attenuated or inactivated, or dead. It will be appreciated by those with skill in this art that certain minor side-effects of vaccination, such as short-term fever, muscle discomfort, general malaise, and other well-known reactions to vaccination using a variety of different types of vaccines, can be anticipated as accompanying vaccination of an animal, preferably a human, using the vaccines of the invention. Such acute, short-term and non-life-threatening side effects are encompassed in the instant definition of the vaccines of the invention, and vaccines causing such side-effects fall within the definition of "attenuated" presented herein. Preferred examples of such attenuated cells include attenutated varieties of *Salmonella* species, preferably *Salmonella typhi* and *Salmonella typhimurium*, as well as other attenuated bacterial species. It will be specifically understood that these embodiments of the vaccines of the invention encompass so-called "live" attenuated cell preparations as well as heat- or chemically-inactivated cell preparations.

In other embodiments of the invention are provided vaccines that are DNA vaccines, comprising the nucleic acids of the invention in recombinant expression constructs competant to direct expression of hemoglobin receptor proteins when introduced into an animal. In preferred embodiments, such DNA vaccines comprise recombinant expression constructs wherein the hemoglobin receptor-encoding nucleic acids of the invention are operably linked to promoter elements, most preferably the early gene promoter of cytomegalovirus or the early gene promoter of simian virus 40. DNA vaccines of the invention are preferably administered by intramuscular injection, but any appropriate route of administration, including oral, transdermal, rectal, nasal, aerosol administration into lung, or any other clinically-acceptable route of administration can be used by those with skill in the art.

In general, the vaccines of the invention are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosage ranges are of the

order of several hundred micrograms active ingredient per individual. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in one or two week intervals by a subsequent injection or other administration.

The recombinant expression constructs of the present invention are also useful in molecular biology to transform bacterial cells which do not ordinarily express a hemoglobin receptor protein to thereafter express this receptor. Such cells are useful, *inter alia*, as intermediates for making cell membrane preparations useful for receptor binding activity assays, vaccine production, and the like, and in certain embodiments may themselves be used, *inter alia*, as vaccines or components of vaccines, as described above. The recombinant expression constructs of the present invention thus provide a method for screening potentially useful bactericidal and bacteriostatic drugs at advantageously lower cost than conventional screening protocols. While not completely eliminating the need for ultimate *in vivo* activity and toxicology assays, the constructs and cultures of the invention provide an important first screening step for the vast number of potentially useful bactericidal and bacteriostatic drugs synthesized, discovered or extracted from natural sources each year. In addition, such bactericidal or bacteriostatic drugs would be selected to utilize a nutritional pathway associated with infectious virulence in these types of bacteria, as disclosed in more detail below, thus selectively targeting bacteria associated with the development of serious infections *in vivo*.

Also, the invention provides both functional bacterial hemoglobin receptor proteins, membranes comprising such proteins, cells expressing such proteins, and the amino acid sequences of such proteins. This invention thereby provides sufficient structural and functional activity information to enable rational drug design of novel therapeutically-active antibacterial drugs using currently-available techniques (*see* Walters, "Computer-Assisted Modeling of Drugs", *in* Klegerman & Groves, eds., 1993, Pharmaceutical Biotechnology, Interpharm Press: Buffalo Grove, IL, pp. 165-174).

Nucleic acids and oligonucleotides of the present invention are useful as diagnostic tools for detecting the existence of a bacterial infection in a human, caused by a hemoglobin receptor protein-expressing pathological organism of *Neisseria* species. Such diagnostic reagents comprise nucleic acid hybridization probes of the invention and encompass paired oligonucleotide PCR primers, as described above. Methods provided by the invention include blot hybridization, *in situ* hybridization and *in vitro* amplification techniques for detecting the

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presence of pathogenic bacteria in a biological sample. Appropriate biological samples advantageously screened using the methods described herein include plasma, serum, lymph, cerebrospinal fluid, seminal fluid, mucosal tissue samples, biopsy samples, and other potential sites of bacterial infection. It is also envisioned that the methods of the invention may be used to screen water, foodstuffs, pharmaceuticals, and other potential sources of infection.

The invention also provides antibodies that are immunologically reactive to a bacterial hemoglobin receptor protein or epitopes thereof provided by the invention. The antibodies provided by the invention may be raised, using methods well known in the art, in animals by inoculation with cells that express a bacterial hemoglobin receptor protein or epitopes thereof, cell membranes from such cells, whether crude membrane preparations or membranes purified using methods well known in the art, or purified preparations of proteins, including fusion proteins, particularly fusion proteins comprising epitopes of a bacterial hemoglobin receptor protein of the invention fused to heterologous proteins and expressed using genetic engineering means in bacterial, yeast or eukaryotic cells, said proteins being isolated from such cells to varying degrees of homogeneity using conventional biochemical means. Synthetic peptides made using established synthetic means *in vitro* and optionally conjugated with heterologous sequences of amino acids, are also encompassed in these methods to produce the antibodies of the invention. Animals that are used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals for inoculation are rodents (including mice, rats, hamsters) and rabbits. The most preferred animal is the mouse.

Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell that naturally expresses a bacterial hemoglobin receptor protein as provided by the invention, or any cell or cell line that expresses a bacterial hemoglobin receptor protein of the invention, or any epitope thereof, as a result of molecular or genetic engineering, or that has been treated to increase the expression of an endogenous or heterologous bacterial hemoglobin receptor protein by physical, biochemical or genetic means. Preferred cells are *E. coli* auxotrophic mutant *hemA aroB* cells transformed with a recombinant expression construct of the invention and grown in media supplemented with hemin or hemoglobin as the sole iron (III) source, and cells of *Neisseria* species.

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The present invention also provides monoclonal antibodies that are immunologically reactive with an epitope of a bacterial hemoglobin receptor protein of the invention, or fragment thereof, present on the surface of such cells, preferably *E. coli* cells. Such antibodies are made using methods and techniques well known to those of skill in the art. Monoclonal antibodies provided by the present invention are produced by hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art (*see* Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with a homogeneous preparation of a bacterial hemoglobin receptor protein, membranes comprised thereof, cells expressing such protein, or epitopes of a bacterial hemoglobin receptor protein, used *per se* or comprising a heterologous or fusion protein construct, as described above. The myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from mouse, and the most preferred mouse myeloma cell line is P3X63-Ag8.653. The animals from whom spleens are obtained after immunization are rats, mice and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from *in vitro* cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a mouse, and the monoclonal antibodies obtained from blood and/or ascites fluid.

Monoclonal antibodies provided by the present invention are also produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are immunologically reactive with an epitope of a bacterial hemoglobin receptor protein of the invention. The present invention also encompasses fragments, including but not limited to F(ab) and $F(ab)'_2$ fragments, of such antibody. Fragments are produced by any number of methods, including but not limited to

proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a bacterial hemoglobin receptor protein, made by methods known to those of skill in the art.

The antibodies and fragments used herein can be labeled preferably with radioactive labels, by a variety of techniques. For example, the biologically active molecules can also be labeled with a radionucleotide via conjugation with the cyclic anhydride of diethylenetriamine penta-acetic acid (DPTA) or bromoacetyl aminobenzyl ethylamine diamine tetra-acidic acid (BABE). *See* Hnatowich *et al.* (1983, *Science* 220: 613-615) and Meares *et al.* (1984, *Anal. Biochem.* 142: 68-78, both references incorporated by reference) for further description of labeling techniques.

The present invention also encompasses an epitope of a bacterial hemoglobin receptor protein of the invention, comprised of sequences and/or a conformation of sequences present in the receptor molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of a receptor molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using methods well known to those skilled in the art. The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered prokaryotic or eukaryotic cells.

The invention also includes chimeric antibodies, comprised of light chain and heavy chain peptides immunologically reactive to a bacterial hemoglobin receptor protein-derived epitope. The chimeric antibodies embodied in the present invention include those that are derived from naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

Also provided by the present invention are diagnostic and therapeutic methods of detecting and treating an infection in a human, by a pathogenic organisms expressing a bacterial hemoglobin receptor protein. Diagnostic reagents for use in such methods include the antibodies, most preferably monoclonal antibodies, of the invention. Such antibodies are used in conventional immunological techniques, including but not limited to enzyme-linked immunosorbent assay (ELISA), radioimmune assay (RIA), Western blot assay, immunological

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titration assays, immunological diffusion assays (such as the Ouchterlony assay), and others known to those of skill in the art. Also provided are epitopes derived from a bacterial hemoglobin receptor protein of the invention and immunologically cross-reactive to said antibodies, for use in any of the immunological techniques described herein.

Additional diagnostic assays include nucleic acid hybridization assays, using the nucleic acids of the invention or specifically-hybridizing fragments thereof, for sensitive detection of bacterial genomic DNA and/or mRNA. Such assays include various blot assays, such as Southern blots, Northern blots, dot blots, slot blots and the like, as well as *in vitro* amplification assays, such as the polymerase chain reaction assay (PCR), reverse transcriptase-polymerase chain reaction assay (RT-PCR), ligase chain reaction assay (LCR), and others known to those skilled in the art. Specific restriction endonuclease digestion of diagnostic fragments detected using any of the methods of the invention, analogous to restriction fragment linked polymorphism assays (RFLP) are also within the scope of this invention.

The invention also provides therapeutic methods and reagents for use in treating infections in a human, cause by a microorganism expressing a bacterial hemoglobin receptor protein of the invention, most preferably a bacteria of *Neisseria* species. Therapeutic reagents for use in such methods include the antibodies, most preferably monoclonal antibodies, of the invention, either *per se* or conjugated to bactericidal or bacteriostatic drugs or other antibiotic compounds effective against the infectious microorganism. In such embodiments, the antibodies of the invention comprise pharmaceutical compositions, additionally comprising appropriate pharmaceutically-acceptable carriers and adjuvants or other ancillary components where necessary. Suitable carriers are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the pharmaceutical formulation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or other compounds which enhance the effectiveness of the antibody. In these embodiments, it will be understood that the therapeutic agents of the invention serve to target the infectious bacteria, either by immunologically "tagging" the bacteria with an antibody of the invention for recognition by cytotoxic cells of a human's immune system, or by specifically delivering an antimicrobial drug to the infectious microorganism *via* the bacterial hemoglobin receptor protein.

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Additional therapeutic reagents include the nucleic acids of the invention or fragments thereof, specifically antisense embodiments of such nucleic acids. Such antisense nucleic acids may be used themselves or embodied in a recombinant expression construct specific for antisense expression, wherein said construct is genetically engineered to co-opt a portion of the genome of a bacterial virus, preferably a bacteriophage, infectious for the bacterial pathogen responsible for the infection. In these embodiments, introduction of the antisense nucleic acids of the invention into the bacterial cell inhibits, attentuates or abolishes expression of the bacterial hemoglobin receptor, thereby reducing the virulence of the bacterial infection and enabling more effective antibacterial interventions. In additional embodiments, bacteriophage are provided bearing "knockout" copies of a bacterial hemoglobin receptor gene, whereby the phage achieves genetic mutation of the endogenous hemoglobin receptor gene in the infectious bacteria *via*, *for example*, homologous recombination of the exogenous knockout copy of the bacterial hemoglobin receptor gene with the endogenous hemoglobin receptor gene in the infectious microorganism.

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Plasmids, bacteria, and media

Plasmids and bacteria used herein are listed on Table 1. *E. coli* strains were routinely grown in Luria-Bertani (LB) broth supplemented with 5-aminolevulinic acid and 50mg/L hemin chloride as necessary. *N. meningitidis* 8013 is a serogroup C clinical isolate (Nassif *et al.*, 1993, *Mol. Microbiol.* 8: 719-725). The meningococci were routinely grown on GCB agar (Difco) supplemented as described by Kellogg *et al.* (1963, *J. Bacteriol* 85: 1274-1279), and incubated at 37°C under a 5% CO_2 atmosphere. Transformation of meningococci was performed as described by Nassif *et al.* (1992, *Mol. Microbiol.* 6: 591-597). When necessary, the following antibiotics were used with *E. coli*: rifampicin, 100 mg/L; tetracycline, 15 mg/L; kanamycin, 30 mg/L; chloramphenicol, 20 mg/L; carbenicillin, 100 mg/L. For *Neisseriae*, kanamycin at 100 mg/L was used when needed.

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EXAMPLE 2

Auxotroph Complementation Cloning of a hemoglobin Receptor Gene from *Neisseria meningitidis*

In order to identify *N. meningitidis* outer membrane receptor(s) involved in the uptake of haemin and/or haemoglobin iron, an auxotroph complementation cloning strategy was used, similar to the approach previously taken to identify the *Y. enterocolitica* and *V. cholerae* hemin receptors (*see* Stojiljkovic and Hantke, 1992, *EMBO J.* 11: 4359-4367; Henderson and Payne, 1994, *J. Bacteriol.* 176: 3269-3277). This strategy is based on the fact that the outer membrane of Gram-negative bacteria is impermeable to hemin (McConville and Charles, 1979, *J. Microbiol.* 113: 165-168) and therefore *E. coli* porphyrin biosynthesis mutants cannot grow on exogenously supplied hemin. If provided with the *N. meningitidis* outer membrane hemin receptor gene, the *E. coli* porphyrin mutant would be able to use exogenously supplied hemin as its porphyrin source.

A cosmid bank of *N. meningitidis* 8013 clone 6 DNA was prepared using conventional cosmid cloning methodologies (Sambrook *et al.*, 1989, *ibid.*). *N. meningitidis* bacterial DNA was partially digested by *MboI*, size fractionated on sucrose gradients and cloned into the *Bam*HI site of the cosmid vector pLAFR2 (Riboli *et al.*, 1991, *Microb. Pathogen.* 10: 393-403). This cosmid bank was mobilized into the *E. coli hemA aroB Rif*^{r} recipient strain by triparental matings using a conjugal plasmid pRK2013::Tn*9*. The mating mixture was plated onselective plates containing hemin chloride (50mg/L), 0.1 mM 2,2'-dypyridil and rifampicin (100 mg/L). Several clones growing on exogenously supplied haemin were isolated after an overnight incubation.

The hemin utilization phenotype of these transformants was tested by re-introduction of the cosmids into naive *E. coli hemA aroB* cells and by monitoring the growth on hemin-supplemented plates. The ability of *E. coli* strains to utilize heme or hemoglobin as the sole iron source was tested as previously described (Stojiljkovic and Hantke, 1992, *ibid.*). Cells were grown on LB agar supplemented with 50μM deferoxamine mesylate (an iron chelating agent, obtained from Sigma Chemical Co., St. Louis, MO). Filter discs (1/4 inches, Schleichner & Schuell, Inc., Keene, NH.) impregnated with the test compounds (20 μL of 5 mg/ml stock solutions unless otherwise stated) were placed on these plates. After overnight growth at 37°C

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TABLE I

STRAIN	GENOTYPE
E. coli K12	
EB53	*hemA*, *aroB*, *rpoB*
KP1041	MC4100*tonB::Km*^{r}
H1388	*exbB::*Tn*10* Δ*lac pro*
TSM348	*endA*, *hsdR*, *pro*, *supF*, pRK2013::Tn*9*
IR754	EB53, *tonB::Km*^{r}
IR736	EB53, *exbB::*Tn*10*
DH5α	*recA*, *gyrB*
N. meningitidis	
ATCC 13077	Serotype A
--	Serotype B*
MC8013	clone 6, wild type
MC*hmbR*	*hmbR::aphA-3*
N. gonorrhoeae MS11A	
PLASMIDS	
pSUSK	pA15 replicon, chloramphenicol^r
pHEM22	pLAFR2, hemoglobin-utilizing cosmid
pHEM44	pLAFR2, hemin-utilizing cosmid
pIRS508	6kb *Cla*I, pSUSK
pIRS523	3kb *Bam*HI/*Sal*I, pUC19
pIRS525	1.2kb *aphA-3*, in *Not*I site of pIRS523
pIRS527	4kb *Bam*HI/*Cla*I, pBluescript
pIRS528	0.7kb *Not*I/*Bam*HI, pBluescript
pIRS692	3.3kb *Bam*HI/*Hin*dIII, SU(SK)

* Laboratory collection

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with 5% CO_2, zones of growth around the discs were monitored. The iron-bound proteins tested in this assay (all obtained from Sigma Chemicals Co.) were hemoglobin from human, baboon, bovine and mouse sources, bovine hemin, human lactoferrin (90% iron saturated), and human transferrin (90% iron saturated, obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN). A total of six hemin utilization positive cosmids were obtained using this protocol. Results using such assays are shown in Table II.

EXAMPLE 3

Restriction Enzyme Digestion Mapping of Hemin Utilization Positive Cosmids

Cosmid DNA from six hemin-utilization positive cosmids obtained as described in Example 2 were digested with *Cla*I, and the resulting fragments were cloned into *Cla*I-digested pSU(SK) vector (obtained from Stratagene, LaJolla, CA). One subclone, containing a 6 kb *Cla*Ifragment from cosmid cos22 (the resultant plasmid was designated pIRS508), was determined to allow utilization of hemin and hemoglobin by *E. coli hemA aroB* assayed as described in Example 2. Another such clone, containing an 11 kb *Cla*I fragment from cos44 was also determined to allow hemin utilization in these auxotrophic mutant cells. Restriction analysis and Southern hybridization indicated that the DNA fragments originating from cos22 and cos44 are unrelated.

The deduced restriction enzyme digestion map of cosmid clone pIRS508 is shown in Figure 1. Plasmid pIRS508 enabled *E. coli hemA aroB* to use both hemin and bovine hemoglobin as iron sources although growth on hemoglobin was somewhat weaker than on hemin (Table II). Further subcloning localized the hemin/hemoglobin utilization locus to the *Bam*HI/*Hin*dIII fragment of the insert. In addition to sequences encoding the hemoglobin receptor gene (designated *hmbR*), sequences for a *Neisseria* insertion element (IS*1106*) and a portion of a *Neisseria* small repetitive element (IR*1*) are also represented in the Figure.

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Table II

STRAIN	ϕ-TYPE	HEMIN IRON	PORPHYRIN	Hb IRON
N. meningitidis				
MC8013	wild type	+++	N.T.	+++
MC*hmbR*	Hb^R mutant	+++	N.T.	-
E. coli				
EB53	iron utilization$^-$	-	-	-
EB53 (pIRS508)	*tonB*$^+$,*exbB*$^+$,*hmbR*$^+$	+++	+++	+
IR754(pIRS508)	*tonB*$^-$,*exbB*$^+$,*hmbR*$^+$	-	-	-
IR736(pIRS508)	*tonB*$^+$,*exbB*$^-$,*hmbR*$^+$	-	-	-

N.T.-not tested. Use of hemin/hemoglobin as a porphyrin source was tested by scoring for growth of strains around hemin (5mg/mL) or hemoglobin (for *E. coli*, 10 mg/mL; for *N. meningitidis*, 5 mg/mL) discs on LB plates. The use of the hemin/hemoglobin as an iron source was tested similarly except NBD plates supplemented with 50 μL of 5 g/L delta-aminolevulinic acid were used (GCB plates supplemented with the 50μM Desferal in the case of *N. meningitidis*).

-: indicates no growth; +: less then 100 mm of growth zone around the disc: +++: $\pm$15 mm of growth zone around the disc.

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EXAMPLE 4
Nucleotide Sequence Analysis of a Cosmid Clone Encoding a *Neisseria* Hemoglobin Receptor Gene

The nucleotide sequence of the 3.3 kb *Bam*HI-*Hind*III DNA fragment carrying the *hmbR* gene and its promoter region was determined using the dideoxy chain termination method using a Sequenase 2.0 kit (obtained from U.S. Biochemicals, Cleveland, OH) and analyzed using a BioRad electrophoresis system, an AutoRead kit (obtained from Pharmacia, Uppsala, SE) and an ALF-370 automatic sequenator (Pharmacia, Uppsala, Sweden). Plasmid subclones for sequencing were produced by a nested deletion approach using Erase-a-Base kit (obtained from Promega Biotech, Madison, WI) using different restriction sites in the *hmbR* gene. The nucleotide and predicted amino acid sequences of the *hmbR* gene are shown in Figure 2

An open reading frame (ORF) encoding the *N. meningitidis*, serotype C hemoglobin receptor protein begins at position 470 of the sequence and encodes a protein having an amino acid sequence of 792 amino acids, with a calculated molecular weight of 85.5 kDa. A Shine-Delgarno sequence (SD) is found at position 460. The HmbR receptor protein contains a signal peptidase I recognition sequence at residues 22 to 24 of the protein (underlined), consistent with the fact that it is an outer membrane protein.

A typical Fur binding nucleotide sequence (designated "Fur box") was found in the promoter region of the *hmbR* gene (Figure 2). Like hemin utilization in *Yersiniae* and *Vibrio*, hemin and hemoglobin utilization in *Neisseria* are known to be iron-inducible phenotypes (West and Sparling, 1985, *Infect. Immun.* 47: 388-394; Dyer *et al.*, 1987, *Infect. Immun.* 55: 2171-2175). In Gram-negative bacteria, conditional expression of many iron utilization genes is regulated by the Fur repressor, which recognizes a 19 bp imperfect dyad repeat (Fur-box) in the promoter regions of Fur-repressed genes. Recently, a genetic screen (FURTA) for the identification of Fur-regulated genes from different Gram-negative bacteria was described (Stojiljkovic *et al.*, 1994, *J. Mol. Biol.* 236: 531-545), and this assay was used to test whether *hmbR* expression was controlled in this way. Briefly, a plasmid carrying a Fur-box sequence is transformed into an *E. coli* strain (H1717) which possesses a Fur-regulated *lac* fusion in the chromosome. Expression of this Fur-regulated *lac* fusion is normally repressed. Introduction of a multicopy Fur-box sequence on the plasmid titrates the available Fur repressor thus allowing

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expression of the Fur-regulated *lac* fusion (this phenotype is termed FURTA positive). Using this screen, the smallest insert fragment from cosmid pIRS508 that produced a FURTA positive result was a 0.7 kb *Bam*HI-*Not*I DNA fragment carried on plasmid pIRS528 (*see* Figure 1). This result indicated that the 0.7 kb *Bam*HI-*Not*I fragment carries a Fur-box and that gene expression from the *hmbR* promoter is controlled by a fur-type operon.

N. meningitidis, serotype C hemoglobin receptor protein was expressed *in vitro* using an *E. coli* S30 extract system from Promega Biotech (Madison, WI). The 3.3 kb *Bam*HI-*Hind*III fragment, expressed *in vitro*, encoded a 90kDa protein which corresponds in size to the predicted molecular weight of the unprocessed HmbR receptor. SDS/ 10% PAGE analysis showing the observed M_r of 90K is shown in Figure 3.

Immediately downstream of the *hmbR* gene (at positions 2955 to 3000 bp in Figure 2) was found a short nucleotide sequence that is 99% identical to the flanking sequence of the PIII gene of *N. gonorrhoeae* (Gotschlich *et al.*, 1987, *J. Exp. Med.* 165: 471-482). The first 26 bp of this sequence represents one half of the inverted repeat (IR1) of the *N. gonorrhoeae* small repetitive element. This element is found in approximately 20 copies in both *N. gonorrhoeae* and *N. meningitidis* (Correia *et al.*, 1988, *J. Biol. Chem.* 263: 12194-12198). The analysis of the nucleotide sequence from position 3027 to the *Cla*I (3984) restriction site (only the nucleotide sequence from *Bam*HI (1) to *Hin*dIII (3370) is shown in Figure 2) indicated the presence of an IS1106 element (Knight *et al.*, 1992, *Mol. Microbiol.* 6: 1565-1573). Interestingly, no nucleotide sequence similar to the IS1106 inverted repeat was found between the IR1 element and the beginning of the homology to IS1106.

These results were consistent with the cloning and identification of a novel hemoglobin receptor protein gene from *N. meningitidis*, embodied in a 3.3kb *Bam*HI/*Hin*dIII fragment of *N. meningitidis* genomic DNA.

EXAMPLE 5

Amino Acid Sequence Comparison of the *N. meningitidis* Hemoglobin Receptor Protein and *Neisseria* Lactoferrin and Transferrin Receptor Proteins

A comparison of the transferrin (Tbp1; Legrain *et al.*, 1993, *Gene* 130: 81-90), lactoferrin (LbpA; Pettersson *et al.*, 1993, *Infect. Immun.* 61: 4724-4733, and 1994, *J.*

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Bacteriol. 176: 1764-1766) and hemoglobin receptors (HmbR) from *N. meningitidis* is shown in Figure 4. The comparison was done with the CLASTAL program from the PC/GENE program package (Intelligenetics, Palo Alto, CA). Only the amino-terminal and carboxyl terminal segments of the proteins are shown. An asterisk indicates identity and a point indicates similarity at the amino acid level. Lactoferrin and transferrin receptors were found to share 44.4% identity in amino acid sequence. In contrast, homology between these proteins and the hemoglobin receptor disclosed herein was found to be significantly weaker (22% amino acid sequence identity with lactoferrin and 21% with transferrin receptor).

EXAMPLE 6

TonB/ExbBD-Dependence of Hemin Transport by the *N. meningitidis* Hemoglobin Receptor

It was known that the transport of iron-containing siderophores, some colicins and vitamin B12 across the outer membrane of *E. coli* depends on three cytoplasmic membrane proteins: TonB, ExbB and ExbD (Postle 1990, *Mol. Microbiol.* 133: 891-898; Braun and Hantke, 1991, *in* Winkelmann, (ed.), Handbook of Microbial Iron Chelates, CRC Press, Boca Raton, Fla., pp. 107-138). In *Yersinia* and *Hemophilus*, hemin uptake was shown to be a TonB-dependent process (Stojiljkovic and Hantke, 1992, *ibid.*; Jarosik *et al.*, 1994, *Infect. Immun.* 62: 2470-2477). Through direct interaction between the outer membrane receptors and the TonB cytoplasmic machinery, the substrate bound to the receptor is internalized into the periplasm (Heller *et al.*, 1988, *Gene* 64: 147-153; Schoffler and Braun, 1989, *Molec. Gen. Genet.* 217: 378-383). This direct interaction has been associated with a particular amino acid sequence in membrane proteins associated with the TonB machinery.

All TonB-dependent receptors in Gram-negative bacteria contain several regions of high homology in their primary structures (Lundrigan and Kadner, 1986, *J. Biol. Chem.* 261: 10797-10801). In the amino acid sequence comparison described in Example 5, putative TonB-boxes of all three proteins are underlined. The carboxyl terminal end of the HmbR receptor contains the highly conserved terminal phenylalanine and position 782 arginine residues thought to be part of an outer membrane localization signal (Struyve *et al.*, 1991, *J. Mol. Biol.* 218: 141-148; Koebnik, 1993, *Trends Microbiol.* 1: 201). At residue 6 of the mature HmbR protein, an amino

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acid sequence - ETTPVKA - is similar in sequence to the so called TonB-boxes of several Gram-negative receptors (Heller *et al.*, 1988, *ibid.*). Interestingly, the putative TonB-box of HmbR has more homology to the TonB-box of the *N. gonorrhoeae* transferrin receptor (Cornelissen *et al.*, 1992, *J. Bacteriol.* 174: 5788-5797) than to the TonB-boxes of *E. coli* siderophore receptors. When the sequence of the HmbR receptor was compared with other TonB-dependent receptors, the highest similarity was found with *Y. enterocolitica* HemR receptor although the similarity was not as high as to the *Neisseria* receptors.

In order to prove the TonB-dependent nature of the *N. meningitidis*, serotype C hemoglobin receptor, *hmbR* was introduced into *exbB* and *tonB* mutants of *E. coli* EB53, and the ability of the strains to utilize hemin and hemoglobin as porphyrin and iron sources was assessed. In these assays, both mutants of *E. coli* EB53 were unable to use hemin either as a porphyrin source or as an iron source in the presence of a functional *hmbR* (Table 2). The usage of hemoglobin as an iron source was also affected (Table 2). These results are consistent with the notion that the *hmbR* gene product, the *N. meningitidis* hemoglobin receptor protein of the invention, is TonB-dependent, since expression of this gene in TonB wild type *E. coli* supported the use of hemin and hemoglobin as sole iron source in the experiments disclosed in Example 2.

EXAMPLE 7

Functional Demonstration that the *hmbR* Gene Product is the Hemoglobin Receptor Protein in *N. meningitidis*

As shown in the data presented in Table II, *hmbR* mediated both hemin and hemoglobin utilization when expressed in *E. coli*, but hemoglobin utilization was less vigorous than hemin utilization. To determine if the HmbR receptor has the same specificity in *N. meningitidis*, *hmbR* was inactivated with a 1.2kb kanamycin cassette (*aphA-3;* Nassif *et al.*, 1991, *ibid.*) and transformed into wild-type *N. meningitidis* 8013 clone 6 (serotype C) cells. The inactivation of the chromosomal *hmbR* copy of the Km-resistant transformants was confirmed by Southern hybridization, as shown in Figure 5. As can be seen from Figure 5, wild-type *N. meningitidis* genomic DNA contains only one copy of the *hmbR* gene (lanes 1 and 3). In the Km^r transformants, the size of the DNA fragments containing the wild-type gene has increased by

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1.2 kb, which is the size of the Kan cassette (Figure 5, lanes 2 and 4). When tested for its ability to utilize different iron-containing compounds, these mutant cells were found to be unable to use hemoglobin-bound iron, regardless of the source (human, bovine, baboon, mouse). The ability of the mutant to utilize hemoglobin-haptoglobin was not tested because the wild-type *N. meningitidis* strain is unable to use haptoglobin-haemoglobin complex as an iron source. However, the mutant was still able to use hemin iron, lactoferrin- and transferrin-bound iron as well as citrate-iron (Table II). As the iron-containing component of hemoglobin is hemin, a hemoglobin receptor would be expected to be capable of transporting hemin into the periplasm. Indeed, the cloning strategy disclosed herein depended on the ability of the cloned meningococcal receptor to transport hemin into the periplasm of *E. coli*. These results strongly suggest that *N. meningitidis* has at least two functional receptors that are involved in the internalization of hemin-containing compounds. One is the hemoglobin receptor described herein, which allows the utilization of both hemin and hemoglobin as iron sources. The other putative receptor in *N. meningitidis* is a hemin receptor which allows utilization of only hemin. This schema is also consistent with the isolation of several cosmid clones that allow *E. coli* EB53 to utilize hemin. DNAs from these cosmids do not hybridize with our *hmbR* probe , indicating that these clones encode a structurally-distinct receptor protein capable of transporting hemin into the periplasm of *N. meningitidis* cells.

EXAMPLE 8

Attenuation of Virulence in *hmbR* Mutant *N. meningitidis* Cells *In Vivo*

In order to test the importance of hemoglobin and hemin scavenging systems of *N. meningitidis in vivo*, the *hmbR* -mutant and the wild type strain of *N. meningitidis*, serotype C were inoculated into 5 day old infant rats and the numbers of bacteria recovered from blood and cerebrospinal fluid were followed. In these experiments, the method for the assessing *N. meningitidis*, serotype C virulence potential was essentially the same as described by Nassif *et al.* (1992, *ibid.*) using infant inbred Lewis rats (Charles River, Saint Aubin les Elbeufs, France). Inbred rats were used to minimize individual variations. Briefly, the 8013 strain was reactivated by 3 animal passages. After the third passage, bacteria were kept frozen in aliquots at -80° C.

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To avoid the possibility that modifications in the course of infection could result from selection of one spontaneous avirulent variant, one aliquot from the animal-passed frozen stock of 8013 was transformed with chromosomal DNA from the *hmbR* mutant, the resultant Kan^r transformants were pooled without further purification and kept frozen at -80°C. For each experiment, all infant rats were from the same litter. *N. meningitidis* 8013 was grown overnight and 2 X 10^6 bacteria injected intraperitoneally into the infant rat. Three rats were used for each meningococcal strain. The course of infection was followed over a 24 hours time period with blood collected at the indicated times. At the 24 h time period, the rats were sacrificed, the cerebrospinal fluid (CSF) collected and the number of colony-forming units (CFU) determined. Each experiment was performed in replicate; similar results were obtained both times.

The results of these experiments are shown in Figure 6. The *hmbR* - strain, which is unable to use hemoglobin as an iron source, was recovered from the blood of infected animals in significantly lower numbers when compared with the wild type strain. Both the mutant and the wild type strain were still able to cross the blood-brain barrier as indicated by the isolation of bacteria from the cerebrospinal fluid. These results indicate that hemoglobin represents an important iron source for *N. meningitidis* during growth *in vivo*.

EXAMPLE 9

Polymerase Chain Reaction Amplification of Hemoglobin Receptor Genes from *N. meningitidis* Serotypes and *N. gonorrhoeae*

From the nucleotide sequence of the 3.3 kb *Bam*HI-*Hind*III DNA fragment carrying the *hmbR* gene and its promoter region was determined specific oligonucleotide promers for *in vitro* amplification of the homologous hemoglobin receptor protein genes from *N. meningitidis* serotypes A and B and *N. gonorrhoeae* MS11A as follows.

The following oligonucleotide primers were developed for *in vitro* amplificaiton reactions using the polymerase chain reaction (PCR; Saiki *et al.*, 1988, *Science* 230: 1350-1354):

5′-AAACAGGTCTCGGCATAG-3′ (sense primer) (SEQ ID No.:11)

5′-CGCGAATTCAAACAGGTCTCGGCATAG-3′ (antisense primer) (SEQ ID No.:12)

for amplifying the hemoglobin receptor protein from *N. meningitidis*, serotype A;

5′-CGCGAATTCAAAAACTTCCATTCCAGCGATACG-3′ (sense primer) (SEQ ID No.:13)

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5'-TAAAACTTCCATTCCAGCGATACG-3' (antisense primer) (SEQ ID No.:14)

for amplifying the hemoglobin receptor protein from *N. meningitidis*, serotype B;

5'-AAACAGGTCTCGGCATAG-3' (sense primer) (SEQ ID No.:15)

or

5'-CGCGAATTCAAACAGGTCTCGGCATAG-3' (sense primer) (SEQ ID No.:16)

and

5'-CGCGAATTCAAAAACTTCCATTCCAGCGATACG-3' (antisense primer) (SEQ ID No.:17)

or

5'-TAAAACTTCCATTCCAGCGATACG-3' (antisense primer) (SEQ ID No.:18)

for amplifying the hemoglobin receptor protein from *N. gonorrhoeae* MS11A.

Genomic DNA from *N. meningitidis* serotype A or B or *N. gonorrhoeae* species was prepared using standard techniques (*see* Sambrook, *et al.*, *ibid.*), including enzymatic degradation of bacterial cell walls, protoplast lysis, protease and RNase digestion, extraction with organic solvents such as phenol and/or chloroform, and ethanol precipitation. Crude DNA preparations were also used. An amount (typically, about 0.1μg) of genomic DNA was used for each amplification reaction. A PCR amplification reaction consisted of *Pfu* polymerase (Stratagene, LaJolla, CA) and/or *Taq* polymerase (Boehringer Mannheim, Germany) in the appropriate buffer including about 20picomoles of each amplificaiton primer and 200nanomoles of each deoxynucleoside triphosphate. Amplification reactions were performed according to the following scheme:

First cycle	5 min at 95°C
	2 min at 51°C
	6 min at 72°C
Cycles 2-13	45 sec at 95°C
	35 sec at 49°C
	10 min at 72°C

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Cycles 14-30	25 sec at 95°C
	35 sec at 47°C
	10 min at 72°C

Upon completion of the amplification reaction, DNA fragments were cloned either blunt-ended or, after *Eco*RI digestion, into *Eco*RI digested pSUKS or pWKS30 vectors and transformed into bacteria. Positively-selected clones were then analyzed for the presence of recombinant inserts, which were sequenced as described above in Example 4.

As a result of these experiments, three clones encoding the hemoglobin receptor genes from *N. meningitidis* serotypes A and B and *N. gonorrhoeae* MS11A were cloned and the sequence of these genes determined. The nucleic acid sequence for each of these genes are shown in Figures 7 (*N. meningitidis*, serotype A), 8 (*N. meningitidis*, serotype A) and 9 (*N. gonorrhoeae* MS11A).

The degree of homology between the cloned hemoglobin receptors from the different *N. meningitidis* serotypes and *N. gonorrhoeae* MS11A was assessed by nucleic acid and amino acid sequence comparison, as described in Example 5 above. The results of these comparisons are shown in Figures 10 and 11, respectively. Hemoglobin receptor genes from the three *N. meningitidis* serotypes and *N. gonorrhoeae* MS11A were found to be from 86.5% to 93.4% homologous; the most homologous nucleic acids were *N. meningitidis* serotypes B and C, and the most divergent nucleic acids were *N. meningitidis* serotype B and *N. gonorrhoeae* MS11A (Figure 10 and Table III). Homoglobin receptor proteins from all four *Neisseria* species showed a high degree of homology to the other members of the group, ranging from 87% homology between the hemoglobin receptor proteins from *N. gonorrhoeae* MS11A and *N. meningitidis* serotype B to 93% homology between hemoglobin receptor proteins from *N. meningitidis* serotypes A and B (Figure 11). In this comparison, all four receptors were found to share 84.7% amino acid sequence identity, and up to 11.6% sequence similarity (*i.e.*, chemically-related amino acid residues at homologous sites within the amino acid sequence). The non-conserved amino acids were found clustered in the regions of the amino acid sequence corresponding to the external loops in the predicted topographical structure of the hemoglobin receptor proteins.

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TABLE III

*	A	B	C	MS11
A	X	**92.2%**	**93.0%**	**90.4%**
B	93.3%	X	**93.4%**	**86.5%**
C	93.2%	93%	X	**90.4%**
MS11	91.1%	86.8%	91.4%	X

* The numbers in the upper quadrant of the Table (in **boldface**) represent nucleic acid sequence homology between the different hemoglobin receptor genes of the invention, while the numbers in the lower quadrant of the Table represent amino acid sequence homology between the different hemoglobin receptor proteins

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3319 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 471..2848

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGAACTAGTG GATCCAATTT GGGCGCGGCG TTTTTGTTCA AACACGCCCA AAAACTCGAT      60

TACAACGGCG AACACGGCGC GCGCCACCTC GCTCCGCATC CCGACGGGCC GCGGCAAACA     120

CTGGCGCGCC TTCGTCGAGC ATCTTGAACG CTTTGAACCT GACTCCCGAA GCCGAAGCGG     180

AAGCCATTCA AGGCGCGCGC GAAGCCTTTG CATTCTACAA AGTCGTGTTG CGCGAAACCT     240

TCGGCTTGGC AGCCGATGCC GAAGCCCCCG AAGGTATGAT GCCGCACAGG CACTAAAAAA     300

TAATCGAACC AAATAAACAA GGTCTCGGCA TAGCTGTTTG CAGGGACCTT TAATTACACG     360

GCGCGGCTTT GTTTACATGG ATTACTGTCT TATTAAATAT TAATGATTAT CATAAAATCT     420

ATTATTCGCT AACCGATGGA TGAACAATCC ATACATCTTG AGTTGATAAT ATG AAA         476
                                                          Met Lys
                                                            1

CCA TTA CAA ATG CTC CCT ATC GCC GCG CTG GTC GGC AGT ATT TTC GGC       524
Pro Leu Gln Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile Phe Gly
          5                  10                  15

AAT CCG GTC TTT GCG GCA GAT GAA GCT GCA ACT GAA ACC ACA CCC GTT       572
Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr Pro Val
     20                  25                  30

AAG GCA GAG GTA AAA GCA GTG CGC GGT AAA GGC CAG CGC AAT GCG CCT       620
Lys Ala Glu Val Lys Ala Val Arg Gly Lys Gly Gln Arg Asn Ala Pro
 35                  40                  45                  50

GCG GCT GTG GAA CGC GTC AAC CTT AAC CGT ATC AAA CAA GAA ATG ATA       668
Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu Met Ile
                 55                  60                  65

CGC GAC AAC AAA GAC TTG GTG CGC TAT TCC ACC GAT GTC GGC TTG AGC       716
Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly Leu Ser
            70                  75                  80

GAC AGC GGC CGC CAT CAA AAA GGC TTT GCT GTT CGC GGC GTG GAA GGC       764
Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val Glu Gly
         85                  90                  95

AAC CGT GTC GGC GTG AGC ATA GAC GGC GTA AAC CTG CCT GAT TCC GAA       812
Asn Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp Ser Glu
    100                 105                 110

GAA AAC TCG CTG TAC GCC CGT TAT GGC AAC TTC AAC AGC TCG CGT CTG       860
Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser Arg Leu
115                 120                 125                 130

TCT ATC GAC CCC GAA CTC GTG CGC AAC ATC GAC ATC GTA AAA GGG GCG       908
Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys Gly Ala
                135                 140                 145

GAC TCT TTC AAT ACC GGC AGC GGC GCC TTG GGC GGC GGT GTG AAT TAC       956
Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val Asn Tyr
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150 155 160

CAA ACC CTG CAA GGA CGT GAC TTA CTG TTG CCT GAA CGG CAG TTC GGC 1004
Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln Phe Gly
165 170 175

GTG ATG ATG AAA AAC GGT TAC AGC ACG CGT AAC CGT GAA TGG ACA AAT 1052
Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp Thr Asn
180 185 190

ACC CTC GGT TTC GGC GTG AGC AAC GAC CGC GTG GAT GCC GCT TTG CTG 1100
Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala Leu Leu
195 200 205 210

TAT TCG CAA CGG CGC GGC CAT GAA ACT GAA AGC GCG GGC AAG CGT GGT 1148
Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys Arg Gly
215 220 225

TAT CCG GTA GAG GGT GCT GGT AGC GGA GCG AAT ATC CGT GGT TCT GCG 1196
Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly Ser Ala
230 235 240

CGC GGT ATT CCT GAT CCG TCC CAA CAC AAA TAC CAC AGC TTC TTG GGT 1244
Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe Leu Gly
245 250 255

AAG ATT GCT TAT CAA ATC AAC GAC AAC CAC CGC ATC GGC GCA TCG CTC 1292
Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala Ser Leu
260 265 270

AAC GGT CAG CAG GGG CAT AAT TAC ACG GTT GAA GAG TCT TAC AAC CTG 1340
Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr Asn Leu
275 280 285 290

CTT GCT TCT TAT TGG CGT GAA GCT GAC GAT GTC AAC AGA CGG CGT AAC 1388
Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg Arg Asn
295 300 305

ACC AAC CTC TTT TAC GAA TGG ACG CCG GAA TCC GAC CGG TTG TCT ATG 1436
Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu Ser Met
310 315 320

GTA AAA GCG GAT GTC GAT TAT CAA AAA ACC AAA GTA TCT GCG GTC AAC 1484
Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala Val Asn
325 330 335

TAC AAA GGT TCG TTC CCG ATA GAG GAT TCT TCC ACC TTG ACA CGT AAC 1532
Tyr Lys Gly Ser Phe Pro Ile Glu Asp Ser Ser Thr Leu Thr Arg Asn
340 345 350

TAC AAT CAA AAG GAC TTG GAT GAA ATC TAC AAC CGC AGT ATG GAT ACC 1580
Tyr Asn Gln Lys Asp Leu Asp Glu Ile Tyr Asn Arg Ser Met Asp Thr
355 360 365 370

CGC TTC AAA CGC ATT ACC CTG CGT TTG GAC AGC CAT CCG TTG CAA CTC 1628
Arg Phe Lys Arg Ile Thr Leu Arg Leu Asp Ser His Pro Leu Gln Leu
375 380 385

GGG GGG GGG CGA CAC CGC CTG TCG TTT AAA ACT TTC GCC AGC CGC CGT 1676

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Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Ser Arg Arg
            390                 395                 400

GAT TTT GAA AAC CTA AAC CGC GAC GAT TAT TAC TTC AGC GGC CGT GTT      1724
Asp Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Gly Arg Val
        405                 410                 415

GTT CGA ACC ACC AGC AGT ATC CAG CAT CCG GTG AAA ACC ACC AAC TAC      1772
Val Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr Asn Tyr
    420                 425                 430

GGT TTC TCA CTG TCT GAC CAA ATT CAA TGG AAC GAC GTG TTC AGT AGC      1820
Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser Ser
435                 440                 445                 450

CGC GCA GGT ATC CGT TAC GAT CAT ACC AAA ATG ACG CCT CAG GAA TTG      1868
Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu Leu
                455                 460                 465

AAT GCC GAG TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC AAC ACT      1916
Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn Thr
            470                 475                 480

TAT AAA GGC TGG AGC GGT TTT GTC GGC TTG GCG GCG CAA CTG AAT CAG      1964
Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Asn Gln
        485                 490                 495

GCT TGG CGT GTC GGT TAC GAC ATT ACT TCC GGC TAC CGT GTC CCC AAT      2012
Ala Trp Arg Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val Pro Asn
    500                 505                 510

GCG TCC GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGT AAT TGG CTG      2060
Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn Trp Leu
515                 520                 525                 530

CCC AAT CCC AAC CTG AAA GCC GAG CGC ACG ACC ACC CAC ACC CTC TCT      2108
Pro Asn Pro Asn Leu Lys Ala Glu Arg Thr Thr Thr His Thr Leu Ser
                535                 540                 545

CTG CAA GGC CGC AGC GAA AAA GGT ACT TTG GAT GCC AAC CTG TAT CAA      2156
Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu Tyr Gln
            550                 555                 560

AGC AAT TAC CGC AAT TTC CTG TCT GAA GAG CAG AAG CTG ACC ACC AGC      2204
Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr Thr Ser
        565                 570                 575

GGC GAT GTC AGC TGT ACT CAG ATG AAT TAC TAC TAC GGT ATG TGT AGC      2252
Gly Asp Val Ser Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met Cys Ser
    580                 585                 590

AAT CCT TAT TCC GAA AAA CTG GAA TGG CAG ATG CAA AAT ATC GAC AAG      2300
Asn Pro Tyr Ser Glu Lys Leu Glu Trp Gln Met Gln Asn Ile Asp Lys
595                 600                 605                 610

GCC AGA ATC CGC GGT ATC GAG CTG ACG GGC CGT CTG AAT GTG GAC AAA      2348
Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val Asp Lys
                615                 620                 625
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GTA GCG TCT TTT GTT CCT GAG GGC TGG AAA CTG TTC GGC TCG CTG GGT      2396
Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu Gly
            630                 635                 640

TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TTC ACC CAG      2444
Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Phe Thr Gln
        645                 650                 655

CCG TTG AAA GTG ATT GCC GGT ATC GAC TAT GAA AGT CCG AGC GAA AAA      2492
Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser Glu Lys
    660                 665                 670

TGG GGC GTG TTC TCC CGC CTG ACC TAT CTG GGC GCG AAA AAG GTC AAA      2540
Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Val Lys
675                 680                 685                 690

GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC TGG GGT ACG CCT TTG      2588
Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr Pro Leu
                695                 700                 705

CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT TAT GTG      2636
Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr Val
            710                 715                 720

TTC GAT ATG TAC GGC TTC TAC AAA CCG GTG AAA AAC CTG ACT TTG CGT      2684
Phe Asp Met Tyr Gly Phe Tyr Lys Pro Val Lys Asn Leu Thr Leu Arg
        725                 730                 735

GCA GGC GTA TAT AAT GTG TTC AAC CGC AAA TAC ACC ACT TGG GAT TCC      2732
Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp Asp Ser
    740                 745                 750

CTG CGC GGC CTG TAT AGC TAC AGC ACC ACC AAC TCG GTC GAC CGC GAT      2780
Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ser Val Asp Arg Asp
755                 760                 765                 770

GGC AAA GGC TTA GAC CGC TAC CGC GCC CCA AGC CGT AAT TAC GCC GTA      2828
Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Ser Arg Asn Tyr Ala Val
                775                 780                 785

TCG CTG GAA TGG AAG TTT TA ATCTGGTATT ATTGAATTAA TCGCCTTGTT          2878
Ser Leu Glu Trp Lys Phe
            790

GAAAATTAAA GCCGTCCGAA TTGTGTTCAA GAACTCATTC GGACGGTTTT TACCGAATCT    2938

GTGTGTGGGT TTATAGTGGA TTAACAAAAA TCAGGACAAG GCGACGAAGC CGCAGACAGT    2998

ACAGATAGTA CGGAACCGAT TCACTTGGTG AGACCTTTGC AAAATTCCTT TCCCTCCCGA    3058

CAGCCGAAAC CCAAACACAG GTTTTCGGCT GTTTTCGCCC CAAATACCTC CTAATTCTAC    3118

CCAAATACCC CCTTAATCCT CCCCGATACC CGATAATCAG GCATCCGGCG CCTTTAGGCG    3178

GCAGCGGGCG CACTTAACCT GTTGGCGGCT TTCAAAAGGT TCAAACACAT CGCCTTCAGG    3238

TGCCTTTGCG CACTCACTTT AATCAGTCCG AAATAGGCCG CCCGCGCATA GCAGAACTTA    3298

CGGTGCAGCG TACCGAAGCT T                                              3319
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 792 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Lys Pro Leu Gln Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
  1               5                  10                  15

Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
             20                  25                  30

Pro Val Lys Ala Glu Val Lys Ala Val Arg Gly Lys Gly Gln Arg Asn
         35                  40                  45

Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
     50                  55                  60

Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
 65                  70                  75                  80

Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
                 85                  90                  95

Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
            100                 105                 110

Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
    130                 135                 140

Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
                165                 170                 175

Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190

Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
    210                 215                 220

Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225                 230                 235                 240

Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
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245 250 255

Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
260 265 270

Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
275 280 285

Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
290 295 300

Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305 310 315 320

Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
325 330 335

Val Asn Tyr Lys Gly Ser Phe Pro Ile Glu Asp Ser Ser Thr Leu Thr
340 345 350

Arg Asn Tyr Asn Gln Lys Asp Leu Asp Glu Ile Tyr Asn Arg Ser Met
355 360 365

Asp Thr Arg Phe Lys Arg Ile Thr Leu Arg Leu Asp Ser His Pro Leu
370 375 380

Gln Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Ser
385 390 395 400

Arg Arg Asp Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Gly
405 410 415

Arg Val Val Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr
420 425 430

Asn Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe
435 440 445

Ser Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln
450 455 460

Glu Leu Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala
465 470 475 480

Asn Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu
485 490 495

Asn Gln Ala Trp Arg Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val
500 505 510

Pro Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn
515 520 525

Trp Leu Pro Asn Pro Asn Leu Lys Ala Glu Arg Thr Thr Thr His Thr
530 535 540

Leu Ser Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu
545 550 555 560

```
Tyr Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr
                565                 570                 575

Thr Ser Gly Asp Val Ser Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met
            580                 585                 590

Cys Ser Asn Pro Tyr Ser Glu Lys Leu Glu Trp Gln Met Gln Asn Ile
        595                 600                 605

Asp Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val
    610                 615                 620

Asp Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser
625                 630                 635                 640

Leu Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Phe
                645                 650                 655

Thr Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser
            660                 665                 670

Glu Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys
        675                 680                 685

Val Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr
    690                 695                 700

Pro Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala
705                 710                 715                 720

Tyr Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Val Lys Asn Leu Thr
                725                 730                 735

Leu Arg Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp
            740                 745                 750

Asp Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ser Val Asp
        755                 760                 765

Arg Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Ser Arg Asn Tyr
    770                 775                 780

Ala Val Ser Leu Glu Trp Lys Phe
785                 790
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2376 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2373

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG AAA CCA TTA CAA ATG CCC CCT ATC GCC GCG CTG CTC GGC AGT ATT       48
Met Lys Pro Leu Gln Met Pro Pro Ile Ala Ala Leu Leu Gly Ser Ile
  1               5                  10                  15

TTC GGC AAT CCG GTC TTT GCG GCA GAT GAA GCT GCA ACT GAA ACC ACA       96
Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
             20                  25                  30

CCC GTT AAG GCA GAG GTA AAA GCA GTG CGC GTT AAA GGT CAG CGC AAT      144
Pro Val Lys Ala Glu Val Lys Ala Val Arg Val Lys Gly Gln Arg Asn
         35                  40                  45

GCG CCT GCG GCT GTG GAA CGC GTC AAC CTT AAC CGT ATC AAA CAA GAA      192
Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
     50                  55                  60

ATG ATA CGC GAC AAT AAA GAC TTG GTG CGC TAT TCC ACC GAT GTC GGC      240
Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
 65                  70                  75                  80

TTG AGC GAC AGG AGC CGT CAT CAA AAA GGC TTT GCC ATT CGC GGC GTG      288
Leu Ser Asp Arg Ser Arg His Gln Lys Gly Phe Ala Ile Arg Gly Val
                 85                  90                  95

GAA GGC GAC CGT GTC GGC GTT AGT ATT GAC GGC GTA AAC CTG CCT GAT      336
Glu Gly Asp Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
            100                 105                 110

TCC GAA GAA AAC TCG CTG TAC GCC CGT TAT GGC AAC TTC AAC AGC TCG      384
Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

CGT CTG TCT ATC GAC CCC GAA CTC GTG CGC AAC ATC GAC ATC GTA AAA      432
Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
    130                 135                 140

GGG GCG GAC TCT TTC AAT ACC GGC AGC GGC GCC TTG GGC GGC GGT GTG      480
Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

AAT TAC CAA ACC CTG CAA GGA CGT GAC TTA CTG TTG CCT GAA CGG CAG      528
Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
                165                 170                 175

TTC GGC GTG ATG ATG AAA AAC GGT TAC AGC ACG CGT AAC CGT GAA TGG      576
Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190
```

```
ACA AAT ACC CTC GGT TTC GGC GTG AGC AAC GAC CGC GTG GAT GCC GCT       624
Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

TTG CTG TAT TCG CAA CGG CGC GGC CAT GAA ACT GAA AGC GCG GGC AAG       672
Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
    210                 215                 220

CGT GGT TAT CCG GTA GAG GGT GCT GGT AGC GGA GCG AAT ATC CGT GGT       720
Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225                 230                 235                 240

TCT GCG CGC GGT ATT CCT GAT CCG TCC CAA CAC AAA TAC CAC AGC TTC       768
Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
                245                 250                 255

TTG GGT AAG ATT GCT TAT CAA ATC AAC GAC AAC CAC CGC ATC GGC GCA       816
Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
            260                 265                 270

TCG CTC AAC GGT CAG CAG GGG CAT AAT TAC ACG GTT GAA GAG TCT TAC       864
Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
        275                 280                 285

AAC CTG CTT GCT TCT TAT TGG CGT GAA GCT GAC GAT GTC AAC AGA CGG       912
Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
    290                 295                 300

CGT AAC ACC AAC CTC TTT TAC GAA TGG ACG CCG GAA TCC GAC CGG TTG       960
Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305                 310                 315                 320

TCT ATG GTA AAA GCG GAT GTC GAT TAT CAA AAA ACC AAA GTA TCT GCG      1008
Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
                325                 330                 335

GTC AAC TAC AAA GGT TCG TTC CCG ACG AAT TAC ACC ACA TGG GAA ACC      1056
Val Asn Tyr Lys Gly Ser Phe Pro Thr Asn Tyr Thr Thr Trp Glu Thr
            340                 345                 350

GAG TAC CAT AAA AAG GAA GTT GGC GAA ATC TAT AAC CGC AGC ATG GAT      1104
Glu Tyr His Lys Lys Glu Val Gly Glu Ile Tyr Asn Arg Ser Met Asp
        355                 360                 365

ACA ACC TTC AAA CGT ATT ACG CTG CGT ATG GAC AGC CAT CCG TTG CAA      1152
Thr Thr Phe Lys Arg Ile Thr Leu Arg Met Asp Ser His Pro Leu Gln
    370                 375                 380

CTC GGG GGG GGG CGA CAC CGC CTG TCG TTT AAA ACC TTT GCC GGG CAG      1200
Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Gly Gln
385                 390                 395                 400

CGT GAT TTT GAA AAC TTA AAC CGC GAC GAT TAC TAC TTC AGC GGC CGT      1248
Arg Asp Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Gly Arg
                405                 410                 415

GTT GTT CGA ACC ACC AAC AGT ATC CAG CAT CCG GTG AAA ACC ACC AAC      1296
Val Val Arg Thr Thr Asn Ser Ile Gln His Pro Val Lys Thr Thr Asn
            420                 425                 430
```

TAC GGT TTC TCG CTG TCC GAC CAA ATC CAA TGG AAC GAC GTG TTC AGT 1344
Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser
435 440 445

AGC CGC GCA GGT ATC CGT TAC GAC CAC ACC AAA ATG ACG CCT CAG GAA 1392
Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu
450 455 460

TTG AAT GCC GAC TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC AAC 1440
Leu Asn Ala Asp Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn
465 470 475 480

ACT TAT AAA GGC TGG AGC GGA TTT GTC GGC TTG GCG GCG CAG CTG AGC 1488
Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Ser
485 490 495

CAA ACA TGG CGT TTG GGT TAC GAT GTG ACC TCA GGT TTC CGC GTG CCG 1536
Gln Thr Trp Arg Leu Gly Tyr Asp Val Thr Ser Gly Phe Arg Val Pro
500 505 510

AAT GCG TCT GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGC ACT TGG 1584
Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Thr Trp
515 520 525

AAG CCT AAT CCT AAT TTG AAG GCA GAA CGC AGC ACC ACC CAC ACC CTG 1632
Lys Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr Leu
530 535 540

TCC TTG CAG GGG CGC GGC GAC AAA GGG ACA CTG GAT GCC AAC CTG TAT 1680
Ser Leu Gln Gly Arg Gly Asp Lys Gly Thr Leu Asp Ala Asn Leu Tyr
545 550 555 560

CAA AGC AAT TAC CGA AAC TTC CTG TCG GAA GAG CAG AAT CTG ACT GTC 1728
Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Asn Leu Thr Val
565 570 575

AGC GGC ACA CCC GGC TGT ACT GAG GAG GAT GCT TAC TAC TAT AGA TGC 1776
Ser Gly Thr Pro Gly Cys Thr Glu Glu Asp Ala Tyr Tyr Tyr Arg Cys
580 585 590

AGC GAC CCC TAC AAA GAA AAA CTG GAT TGG CAG ATG AAA AAT ATC GAC 1824
Ser Asp Pro Tyr Lys Glu Lys Leu Asp Trp Gln Met Lys Asn Ile Asp
595 600 605

AAG GCC AGA ATC CGC GGT ATC GAG TTG ACA GGC CGT CTG AAT GTG GAC 1872
Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val Asp
610 615 620

AAA GTA GCG TCT TTT GTT CCT GAG GGT TGG AAA CTG TTC GGC TCG CTG 1920
Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu
625 630 635 640

GGT TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TCC ACA 1968
Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser Thr
645 650 655

CAG CCG CTG AAA GTG ATT GCC GGT ATC GAC TAT GAA AGT CCG AGC GAA 2016
Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser Glu
660 665 670

```
AAA TGG GGC GTA TTC TCC CGC CTG ACC TAT CTA GGC GCG AAA AAG GTC      2064
Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Val
        675                 680                 685

AAA GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC TGG GGT ACG CCT      2112
Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr Pro
    690                 695                 700

TTG CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT TAT      2160
Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr
705                 710                 715                 720

GTG TTT GAT ATG TAC GGC TTC TAC AAA CCG GCT AAA AAC CTG ACT TTG      2208
Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Ala Lys Asn Leu Thr Leu
                725                 730                 735

CGT GCA GGC GTG TAC AAC CTG TTC AAC CGC AAA TAC ACC ACT TGG GAT      2256
Arg Ala Gly Val Tyr Asn Leu Phe Asn Arg Lys Tyr Thr Thr Trp Asp
            740                 745                 750

TCC CTG CGC GGT TTA TAT AGC TAC AGC ACC ACC AAT GCG GTC GAC CGC      2304
Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ala Val Asp Arg
        755                 760                 765

GAT GGC AAA GGC TTA GAC CGC TAC CGC GCC CCA GGC CGC AAT TAC GCC      2352
Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Gly Arg Asn Tyr Ala
    770                 775                 780

GTA TCG CTG GAA TGG AAG TTT TAA                                     2376
Val Ser Leu Glu Trp Lys Phe
785                 790
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 791 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Lys Pro Leu Gln Met Pro Pro Ile Ala Ala Leu Leu Gly Ser Ile
  1               5                  10                  15

Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
            20                  25                  30

Pro Val Lys Ala Glu Val Lys Ala Val Arg Val Lys Gly Gln Arg Asn
        35                  40                  45

Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
    50                  55                  60

Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65                  70                  75                  80
```

Leu Ser Asp Arg Ser Arg His Gln Lys Gly Phe Ala Ile Arg Gly Val
85 90 95

Glu Gly Asp Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
100 105 110

Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
115 120 125

Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
130 135 140

Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145 150 155 160

Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
165 170 175

Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
180 185 190

Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
195 200 205

Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
210 215 220

Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225 230 235 240

Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
245 250 255

Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
260 265 270

Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
275 280 285

Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
290 295 300

Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305 310 315 320

Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
325 330 335

Val Asn Tyr Lys Gly Ser Phe Pro Thr Asn Tyr Thr Thr Trp Glu Thr
340 345 350

Glu Tyr His Lys Lys Glu Val Gly Glu Ile Tyr Asn Arg Ser Met Asp
355 360 365

Thr Thr Phe Lys Arg Ile Thr Leu Arg Met Asp Ser His Pro Leu Gln
370 375 380

Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Gly Gln
385 390 395 400

```
Arg Asp Phe Clu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Gly Arg
                405                 410                 415

Val Val Arg Thr Thr Asn Ser Ile Gln His Pro Val Lys Thr Thr Asn
            420                 425                 430

Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser
        435                 440                 445

Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu
    450                 455                 460

Leu Asn Ala Asp Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn
465                 470                 475                 480

Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Ser
                485                 490                 495

Gln Thr Trp Arg Leu Gly Tyr Asp Val Thr Ser Gly Phe Arg Val Pro
            500                 505                 510

Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Thr Trp
        515                 520                 525

Lys Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr Leu
    530                 535                 540

Ser Leu Gln Gly Arg Gly Asp Lys Gly Thr Leu Asp Ala Asn Leu Tyr
545                 550                 555                 560

Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Asn Leu Thr Val
                565                 570                 575

Ser Gly Thr Pro Gly Cys Thr Glu Glu Asp Ala Tyr Tyr Tyr Arg Cys
            580                 585                 590

Ser Asp Pro Tyr Lys Glu Lys Leu Asp Trp Gln Met Lys Asn Ile Asp
        595                 600                 605

Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val Asp
    610                 615                 620

Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu
625                 630                 635                 640

Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser Thr
                645                 650                 655

Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser Glu
            660                 665                 670

Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Val
        675                 680                 685

Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr Pro
    690                 695                 700
```

```
Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr
705                 710                 715                 720

Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Ala Lys Asn Leu Thr Leu
                725                 730                 735

Arg Ala Gly Val Tyr Asn Leu Phe Asn Arg Lys Tyr Thr Thr Trp Asp
            740                 745                 750

Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ala Val Asp Arg
        755                 760                 765

Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Gly Arg Asn Tyr Ala
    770                 775                 780

Val Ser Leu Glu Trp Lys Phe
785                 790
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2379 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2376

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG AAA CCA TTA CAA ATG CTC CCT ATC GCC GCG CTG GTC GGC AGT ATT        48
Met Lys Pro Leu Gln Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
  1               5                  10                  15

TTC GGC AAT CCG GTC TTT GCG GCA GAT GAA GCT GCA ACT GAA ACC ACA        96
Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
            20                  25                  30

CCC GTT AAG GCA GAG GTA AAA GCA GTG CGC GTT AAA GGC CAG CGC AAT       144
Pro Val Lys Ala Glu Val Lys Ala Val Arg Val Lys Gly Gln Arg Asn
        35                  40                  45

GCG CCT GCG GCT GTG GAA CGC GTC AAC CTT AAC CGT ATC AAA CAA GAA       192
Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
    50                  55                  60

ATG ATA CGC GAC AAC AAA GAC TTG GTG CGC TAT TCC ACC GAT GTC GGC       240
Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65                  70                  75                  80

TTG AGC GAC AGC GGC CGC CAT CAA AAA GGC TTT GCC GTT CGC GGC GTG       288
Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
                85                  90                  95
```

```
GAA GGC AAC CGT GTC GGC GTG AGC ATA GAC GGC GTA AAC CTG CCT GAT        336
Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
            100                 105                 110

TCC GAA GAA AAC TCG CTG TAC GCC CGT TAT GGC AAC TTC AAC AGC TCG        384
Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

CGT CTG TCT ATC GAC CCC GAA CTC GTG CGC AAC ATC GAC ATC GTA AAA        432
Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
    130                 135                 140

GGG GCG GAC TCT TTC AAT ACC GGC AGC GGC GCC TTG GGC GGC GGT GTG        480
Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

AAT TAC CAA ACC CTG CAA GGA CGT GAC TTA CTG TTG CCT GAA CGG CAG        528
Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
                165                 170                 175

TTC GGC GTG ATG ATG AAA AAC GGT TAC AGC ACG CGT AAC CGT GAA TGG        576
Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190

ACA AAT ACC CTC GGT TTC GGC GTG AGC AAC GAC CGC GTG GAT GCC GCT        624
Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

TTG CTG TAT TCG CAA CGG CGC GGC CAT GAA ACT GAA AGC GCG GGC AAG        672
Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
    210                 215                 220

CGT GGT TAT CCG GTA GAG GGT GCT GGT AGC GGA GCG AAT ATC CGT GGT        720
Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225                 230                 235                 240

TCT GCG CGC GGT ATT CCT GAT CCG TCC CAA CAC AAA TAC CAC AGC TTC        768
Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
                245                 250                 255

TTG GGT AAG ATT GCT TAT CAA ATC AAC GAC AAC CAC CGC ATC GGC GCA        816
Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
            260                 265                 270

TCG CTC AAC GGT CAG CAG GGG CAT AAT TAC ACG GTT GAA GAG TCT TAC        864
Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
        275                 280                 285

AAC CTG CTT GCT TCT TAT TGG CGT GAA GCT GAC GAT GTC AAC AGA CGG        912
Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
    290                 295                 300

CGT AAC ACC AAC CTC TTT TAC GAA TGG ACG CCG GAA TCC GAC CGG TTG        960
Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305                 310                 315                 320

TCT ATG GTA AAA GCG GAT GTC GAT TAT CAA AAA ACC AAA GTA TCT GCG       1008
Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
                325                 330                 335
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```
GTC AAC TAC AAA GGT TCG TTC CCG ATA GAG GAT TCT TCC ACC TTG ACA        1056
Val Asn Tyr Lys Gly Ser Phe Pro Ile Glu Asp Ser Ser Thr Leu Thr
            340                 345                 350

CGT AAC TAC AAT CAA AAG GAC TTG GAT GAA ATC TAC AAC CGC AGT ATG        1104
Arg Asn Tyr Asn Gln Lys Asp Leu Asp Glu Ile Tyr Asn Arg Ser Met
        355                 360                 365

GAT ACC CGC TTC AAA CGT ATT ACG CTG CGT TTG GAC AGC CAT CCG TTG        1152
Asp Thr Arg Phe Lys Arg Ile Thr Leu Arg Leu Asp Ser His Pro Leu
    370                 375                 380

CAA CTC GGG GGG GGG CGA CAC CGC CTG TCG TTT AAA ACT TTC GCC AGC        1200
Gln Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Ser
385                 390                 395                 400

CGC CGT GAT TTT GAA AAC CTA AAC CGC GAC TAT TAC TAC TTC AGC GGC        1248
Arg Arg Asp Phe Glu Asn Leu Asn Arg Asp Tyr Tyr Tyr Phe Ser Gly
                405                 410                 415

CGT GTT GTT CGA ACC ACC AGC AGT ATC CAG CAT CCG GTG AAA ACC ACC        1296
Arg Val Val Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr
            420                 425                 430

AAC TAC GGT TTC TCA CTG TCT GAC CAA ATT CAA TGG AAC GAC GTG TTC        1344
Asn Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe
        435                 440                 445

AGT AGC CGC GCA GGT ATC CGT TAC GAT CAT ACC AAA ATG ACG CCT CAG        1392
Ser Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln
    450                 455                 460

GAA TTG AAT GCC GAG TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC        1440
Glu Leu Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala
465                 470                 475                 480

AAC ACT TAT AAA GGC TGG AGC GGT TTT GTC GGC TTG GCG GCG CAA CTG        1488
Asn Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu
                485                 490                 495

AAT CAG GCT TGG CGT GTC GGT TAC GAC ATT ACT TCC GGC TAC CGT GTC        1536
Asn Gln Ala Trp Arg Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val
            500                 505                 510

CCC AAT GCG TCC GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGT AAT        1584
Pro Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn
        515                 520                 525

TGG CTG CCC AAT CCC AAC CTG AAA GCC GAG CGC ACG ACC ACC CAC ACC        1632
Trp Leu Pro Asn Pro Asn Leu Lys Ala Glu Arg Thr Thr Thr His Thr
    530                 535                 540

CTC TCT CTG CAA GGC CGC AGC GAA AAA GGT ACT TTG GAT GCC AAC CTG        1680
Leu Ser Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu
545                 550                 555                 560

TAT CAA AGC AAT TAC CGA AAT TTC CTG TCT GAA GAG CAG AAG CTG ACC        1728
Tyr Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr
                565                 570                 575
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ACC AGC GGC GAT GTC AGC TGT ACT CAG ATG AAT TAC TAC TAC GGT ATG      1776
Thr Ser Gly Asp Val Ser Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met
            580                 585                 590

TGT AGC AAT CCT TAT TCC GAA AAA CTG GAA TGG CAG ATG CAA AAT ATC      1824
Cys Ser Asn Pro Tyr Ser Glu Lys Leu Glu Trp Gln Met Gln Asn Ile
        595                 600                 605

GAC AAG GCC AGA ATC CGC GGT ATC GAG CTG ACG GGC CGT CTG AAT GTG      1872
Asp Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val
    610                 615                 620

GAC AAA GTA GCG TCT TTT GTT CCT GAG GGC TGG AAA CTG TTC GGC TCG      1920
Asp Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser
625                 630                 635                 640

CTG GGT TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TCC      1968
Leu Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser
                645                 650                 655

ACC CAG CCG TTG AAA GTG ATT GCC GGT ATC GAC TAT GAA AGT CCG AGC      2016
Thr Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser
            660                 665                 670

GAA AAA TGG GGC GTG TTC TCC CGC CTG ACC TAT CTG GGC GCG AAA AAG      2064
Glu Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys
        675                 680                 685

GTC AAA GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC TGG GGT ACG      2112
Val Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr
    690                 695                 700

CCT TTG CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT      2160
Pro Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala
705                 710                 715                 720

TAT GTG TTC GAT ATG TAC GGC TTC TAC AAA CCG GTG AAA AAC CTG ACT      2208
Tyr Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Val Lys Asn Leu Thr
                725                 730                 735

TTG CGT GCA GGC GTA TAT AAT GTG TTC AAC CGC AAA TAC ACC ACT TGG      2256
Leu Arg Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp
            740                 745                 750

GAT TCC CTG CGC GGC CTG TAT AGC TAC AGC ACC ACC AAC TCG GTC GAC      2304
Asp Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ser Val Asp
        755                 760                 765

CGC GAT GGC AAA GGC TTA GAC CGC TAC CGC GCC CCA AGC CGT AAT TAC      2352
Arg Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Ser Arg Asn Tyr
    770                 775                 780

GCC GTA TCG CTG GAA TGG AAG TTT TAA                                 2379
Ala Val Ser Leu Glu Trp Lys Phe
785                 790
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 792 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Lys Pro Leu Gln Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
 1               5                  10                  15

Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
            20                  25                  30

Pro Val Lys Ala Glu Val Lys Ala Val Arg Val Lys Gly Gln Arg Asn
        35                  40                  45

Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
    50                  55                  60

Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65                  70                  75                  80

Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
                85                  90                  95

Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
            100                 105                 110

Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
    130                 135                 140

Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
                165                 170                 175

Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190

Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
    210                 215                 220

Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225                 230                 235                 240

Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
                245                 250                 255

Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
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260 265 270

Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
275 280 285

Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
290 295 300

Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305 310 315 320

Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
325 330 335

Val Asn Tyr Lys Gly Ser Phe Pro Ile Glu Asp Ser Ser Thr Leu Thr
340 345 350

Arg Asn Tyr Asn Gln Lys Asp Leu Asp Glu Ile Tyr Asn Arg Ser Met
355 360 365

Asp Thr Arg Phe Lys Arg Ile Thr Leu Arg Leu Asp Ser His Pro Leu
370 375 380

Gln Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Ser
385 390 395 400

Arg Arg Asp Phe Glu Asn Leu Asn Arg Asp Tyr Tyr Tyr Phe Ser Gly
405 410 415

Arg Val Val Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr
420 425 430

Asn Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe
435 440 445

Ser Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln
450 455 460

Glu Leu Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala
465 470 475 480

Asn Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu
485 490 495

Asn Gln Ala Trp Arg Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val
500 505 510

Pro Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn
515 520 525

Trp Leu Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr
530 535 540

Leu Ser Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu
545 550 555 560

Tyr Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr

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                565                 570                 575

Thr Ser Gly Asp Val Ser Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met
            580                 585                 590

Cys Ser Asn Pro Tyr Ser Glu Lys Leu Glu Trp Gln Met Gln Asn Ile
        595                 600                 605

Asp Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val
    610                 615                 620

Asp Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser
625                 630                 635                 640

Leu Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser
                645                 650                 655

Thr Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser
            660                 665                 670


Glu Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys
        675                 680                 685

Val Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr
    690                 695                 700

Pro Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala
705                 710                 715                 720

Tyr Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Val Lys Asn Leu Thr
                725                 730                 735

Leu Arg Ala Cly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp
            740                 745                 750

Asp Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ser Val Asp
        755                 760                 765

Arg Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Ser Arg Asn Tyr
    770                 775                 780

Ala Val Ser Leu Glu Trp Lys Phe
785                 790
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2378 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..2370

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG AAA CCA TTA CAC ATG CTT CCT ATT GCC GCG CTG GTC GGC AGT ATT 48
Met Lys Pro Leu His Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
1 5 10 15

TTC GGC AAT CCG GTC TTG GCA GCG GAT GAA GCT GCA ACC GAA ACC ACA 96
Phe Gly Asn Pro Val Leu Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
20 25 30

CCC GTT AAA GCA GAG ATA AAA GAA GTG CGC GTT AAA GAC CAG CTT AAT 144
Pro Val Lys Ala Glu Ile Lys Glu Val Arg Val Lys Asp Gln Leu Asn
35 40 45

GCG CCT GCA ACC GTG GAA CGT GTC AAC CTC GGC CGC ATT CAA CAG GAA 192
Ala Pro Ala Thr Val Glu Arg Val Asn Leu Gly Arg Ile Gln Gln Glu
50 55 60

ATG ATA CGC GAC AAC AAA GAC TTG GTG CGT TAC TCC ACC GAC GTC GGC 240
Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65 70 75 80

TTG AGC GAT AGC GGC CGC CAT CAA AAA GGC TTT GCT GTG CGC GGC GTG 288
Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
85 90 95

GAA GGC AAC CGT GTC GGT GTC AGC ATT GAC GGC GTG AGC CTG CCT GAT 336
Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Ser Leu Pro Asp
100 105 110

TCG GAA GAA AAC TCA CTG TAT GCA CGT TAT GGC AAC TTC AAC AGC TCG 384
Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
115 120 125

CGC CTG TCT ATC GAC CCC GAA CTC GTG CGC AAC ATC GAA ATC GCG AAG 432
Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Glu Ile Ala Lys
130 135 140

GGC GCT GAC TCT TTC AAT ACC GGT AGC GGC GCA TTG GGT GGC GGC GTG 480
Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145 150 155 160

AAT TAC CAA ACC CTG CAA GGA CAT GAT TTG CTG TTG GAC GAC AGG CAA 528
Asn Tyr Gln Thr Leu Gln Gly His Asp Leu Leu Leu Asp Asp Arg Gln
165 170 175

TTC GGC GTG ATG ATG AAA AAC GGT TAC AGC ACG CGC AAC CGC GAA TGG 576
Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
180 185 190

ACA AAT ACA CTC GGT TTC GGT GTG AGC AAC GAC CGC GTG GAT GCC GCT 624
Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
195 200 205

TTG CTG TAT TCG CAA CGT CGC GGT CAT GAG ACC GAA AGC GCG GGC GAG 672
Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Glu

210 215 220

CGT GGC TAT CCG GTA GAG GGT GCT GGC AGC GGA GCA ATT ATC CGT GGT 720
Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Ile Ile Arg Gly
225 230 235 240

TCG TCA CGC GGT ATC CCT GAT CCG TCC AAA CAC AAA TAC CAC AAC TTC 768
Ser Ser Arg Gly Ile Pro Asp Pro Ser Lys His Lys Tyr His Asn Phe
245 250 255

TTG GGT AAG ATT GCT TAT CAA ATC AAC GAC AAG CAC CGC ATC GGC CCA 816
Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Lys His Arg Ile Gly Pro
260 265 270

TCG TTT AAC GGC CAG CAG GGG CAT AAT TAC ACG ATT GAA GAG TCT TAT 864
Ser Phe Asn Gly Gln Gln Gly His Asn Tyr Thr Ile Glu Glu Ser Tyr
275 280 285

AAC CTG ACC GCT TCT TCC TGG CGC GAA GCC GAT GAC GTA AAC AGA CGG 912
Asn Leu Thr Ala Ser Ser Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
290 295 300

CGC AAT GCC AAC CTC TTT TAC GAA TGG ACG CCT GAT TCA AAT TGG CTG 960
Arg Asn Ala Asn Leu Phe Tyr Glu Trp Thr Pro Asp Ser Asn Trp Leu
305 310 315 320

TCG TCT TTG AAG GCG GAT TTC GAT TAT CAG ACA ACC AAA GTG GCG GCG 1008
Ser Ser Leu Lys Ala Asp Phe Asp Tyr Gln Thr Thr Lys Val Ala Ala
325 330 335

GTT AAC AAC AAA GGC TCG TTC CCG ACG GAT TAT TCC ACC TTG ACG CGC 1056
Val Asn Asn Lys Gly Ser Phe Pro Thr Asp Tyr Ser Thr Leu Thr Arg
340 345 350

AAC TAT AAT CAG AAG GAT TTG GAG AAT ATA TAC AAC CGC AGC ATG GAC 1104
Asn Tyr Asn Gln Lys Asp Leu Glu Asn Ile Tyr Asn Arg Ser Met Asp
355 360 365

ACC CGA TTC AAA CGT TTT ACT TTG CGT ATG GAC AGC CAA CCG TTG CAA 1152
Thr Arg Phe Lys Arg Phe Thr Leu Arg Met Asp Ser Gln Pro Leu Gln
370 375 380

CTG GGC GGC CAA CAT CGC TTG TCG CTT AAA ACT TTC GCC AGT CGG CGT 1200
Leu Gly Gly Gln His Arg Leu Ser Leu Lys Thr Phe Ala Ser Arg Arg
385 390 395 400

GAG TTT GAA AAC TTA AAC CGC GAC GAT TAT TAC TTC AGC GAA AGA GTA 1248
Glu Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Glu Arg Val
405 410 415

TCC CGT ACT ACC AGC TCG ATT CAA CAC CCC GTG AAA ACC ACT AAT TAT 1296
Ser Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr Asn Tyr
420 425 430

GGT TTC TCA CTG TCT GAT CAA ATC CAA TGG AAC GAC GTG TTC AGC AGC 1344
Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser Ser
435 440 445

CGT GCA GAT ATC CGT TAC GAT CAT ACC AAA ATG ACG CCT CAG GAA TTG 1392

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Arg Ala Asp Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu Leu
    450                 455                 460

AAT GCC GAG TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC AAT ACT      1440
Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn Thr
465                 470                 475                 480

TAT AAA GGC TGG AGC GGA TTT GTC GGT TTG GCG GCG CAA CTG AAT CAG      1488
Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Asn Gln
                485                 490                 495

GCT TGG CAT GTC GGT TAC GAC ATT ACT TCC GGC TAC CGT GTC CCC AAT      1536
Ala Trp His Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val Pro Asn
            500                 505                 510

GCG TCC GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGT AAT TGG CTG      1584
Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn Trp Leu
        515                 520                 525

CCC AAT CCC AAC CTG AAA GCC GAG CGC AGC ACC ACC CAC ACC CTG TCT      1632
Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr Leu Ser
    530                 535                 540

CTG CAA GGC CGC AGC GAA AAA GGT ACT TTG GAT GCC AAC CTG TAT CAA      1680
Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu Tyr Gln
545                 550                 555                 560

AGC AAT TAC CGA AAC TTC TTG TCT GAA GAG CAG AAG CTG ACC ACC AGC      1728
Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr Thr Ser
                565                 570                 575

GGC GAT GTC GGC TGT ACT CAG ATG AAT TAC TAC TAC GGT ATG TGT AGC      1776
Gly Asp Val Gly Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met Cys Ser
            580                 585                 590

AAT CCT TAT TCC GAA AAA CCG GAA TGG CAG ATG CAA AAT ATC GAT AAG      1824
Asn Pro Tyr Ser Glu Lys Pro Glu Trp Gln Met Gln Asn Ile Asp Lys
        595                 600                 605

GCC CGA ATC CGT GGT CTT GAG CTG ACG GGC CGT CTG AAT GTG ACA AAA      1872
Ala Arg Ile Arg Gly Leu Glu Leu Thr Gly Arg Leu Asn Val Thr Lys
    610                 615                 620

GTA GCG TCT TTT GTT CCT GAG GGC TGG AAA TTG TTC GGC TCG CTG GGT      1920
Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu Gly
625                 630                 635                 640

TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TCC ACA CAG      1968
Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser Thr Gln
                645                 650                 655

CCG CCG AAA GTG ATT GCC GGT ATC GAC TAT GAA AGT CCG AGC GAA AAA      2016
Pro Pro Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser Glu Lys
            660                 665                 670

TGG GGT GTG TTC TCC CGC CTG ACT TAT CTG GGT GCG AAA AAG GTC AAA      2064
Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Val Lys
        675                 680                 685
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GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC CGG GGT ACG CCT TTG 2112
Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Arg Gly Thr Pro Leu
690 695 700

CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT TAT GTG 2160
Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr Val
705 710 715 720

TTT GAT ATG TAC GGC TTC TAC AAA CTG GCT AAA AAC CTG ACT TTG CGT 2208
Phe Asp Met Tyr Gly Phe Tyr Lys Leu Ala Lys Asn Leu Thr Leu Arg
725 730 735

GCA GGC GTA TAT AAT GTG TTC AAC CGC AAA TAC ACC ACT TGG GAT TCC 2256
Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp Asp Ser
740 745 750

CTG CGC GGT TTG TAT AGC TAC ACC ACC ACC AAC GCG GTC GAC CGA GAT 2304
Leu Arg Gly Leu Tyr Ser Tyr Thr Thr Thr Asn Ala Val Asp Arg Asp
755 760 765

GGC AAA GGC TTA GAC CGC TAC CGC GCC TCA GGC CGT AAT TAC GCC GTA 2352
Gly Lys Gly Leu Asp Arg Tyr Arg Ala Ser Gly Arg Asn Tyr Ala Val
770 775 780

TCG CTG GAT TGG AAG TTT TGAATTCC 2378
Ser Leu Asp Trp Lys Phe
885 790

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 790 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Lys Pro Leu His Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
1 5 10 15

Phe Gly Asn Pro Val Leu Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
20 25 30

Pro Val Lys Ala Glu Ile Lys Glu Val Arg Val Lys Asp Gln Leu Asn
35 40 45

Ala Pro Ala Thr Val Glu Arg Val Asn Leu Gly Arg Ile Gln Gln Glu
50 55 60

Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65 70 75 80

Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
85 90 95

Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Ser Leu Pro Asp

```
                100                 105                 110

Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Glu Ile Ala Lys
    130                 135                 140

Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

Asn Tyr Gln Thr Leu Gln Gly His Asp Leu Leu Leu Asp Asp Arg Gln
                165                 170                 175

Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190

Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Glu
    210                 215                 220

Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Ile Ile Arg Gly
225                 230                 235                 240

Ser Ser Arg Gly Ile Pro Asp Pro Ser Lys His Lys Tyr His Asn Phe
                245                 250                 255

Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Lys His Arg Ile Gly Pro
            260                 265                 270

Ser Phe Asn Gly Gln Gln Gly His Asn Tyr Thr Ile Glu Glu Ser Tyr
        275                 280                 285


Asn Leu Thr Ala Ser Ser Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
    290                 295                 300

Arg Asn Ala Asn Leu Phe Tyr Glu Trp Thr Pro Asp Ser Asn Trp Leu
305                 310                 315                 320

Ser Ser Leu Lys Ala Asp Phe Asp Tyr Gln Thr Thr Lys Val Ala Ala
                325                 330                 335

Val Asn Asn Lys Gly Ser Phe Pro Thr Asp Tyr Ser Thr Leu Thr Arg
            340                 345                 350

Asn Tyr Asn Gln Lys Asp Leu Glu Asn Ile Tyr Asn Arg Ser Met Asp
        355                 360                 365

Thr Arg Phe Lys Arg Phe Thr Leu Arg Met Asp Ser Gln Pro Leu Gln
    370                 375                 380

Leu Gly Gly Gln His Arg Leu Ser Leu Lys Thr Phe Ala Ser Arg Arg
385                 390                 395                 400

Glu Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Glu Arg Val
                405                 410                 415
```

Ser Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr Asn Tyr
420 425 430

Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser Ser
435 440 445

Arg Ala Asp Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu Leu
450 455 460

Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn Thr
465 470 475 480

Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Asn Gln
485 490 495

Ala Trp His Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val Pro Asn
500 505 510

Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn Trp Leu
515 520 525

Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr Leu Ser
530 535 540

Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu Tyr Gln
545 550 555 560

Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr Thr Ser
565 570 575

Gly Asp Val Gly Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met Cys Ser
580 585 590

Asn Pro Tyr Ser Glu Lys Pro Glu Trp Gln Met Gln Asn Ile Asp Lys
595 600 605

Ala Arg Ile Arg Gly Leu Glu Leu Thr Gly Arg Leu Asn Val Thr Lys
610 615 620

Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu Gly
625 630 635 640

Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser Thr Gln
645 650 655

Pro Pro Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser Glu Lys
660 665 670

Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Val Lys
675 680 685

Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Arg Gly Thr Pro Leu
690 695 700

Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr Val
705 710 715 720

Phe Asp Met Tyr Gly Phe Tyr Lys Leu Ala Lys Asn Leu Thr Leu Arg

```
                725                 730                 735

Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp Asp Ser
            740                 745                 750

Leu Arg Gly Leu Tyr Ser Tyr Thr Thr Thr Asn Ala Val Asp Arg Asp
        755                 760                 765

Gly Lys Gly Leu Asp Arg Tyr Arg Ala Ser Gly Arg Asn Tyr Ala Val
    770                 775                 780

Ser Leu Asp Trp Lys Phe
785                 790
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 600 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Gln Gln Gln His Leu Phe Arg Leu Asn Ile Leu Cys Leu Ser Leu
1               5                   10                  15

Met Thr Ala Leu Pro Val Tyr Ala Glu Asn Val Gln Ala Glu Gln Ala
            20                  25                  30

Gln Glu Lys Gln Leu Asp Thr Ile Val Lys Ala Lys Lys Gln Lys Thr
        35                  40                  45

Arg Arg Asp Asn Glu Val Thr Gly Leu Gly Lys Leu Val Lys Ser Ser
    50                  55                  60

Asp Thr Leu Ser Lys Glu Gln Val Leu Asn Ile Arg Asp Leu Thr Arg
65                  70                  75                  80

Tyr Asp Pro Gly Ile Ala Val Val Glu Gln Gly Arg Gly Ala Ser Ser
                85                  90                  95

Gly Tyr Ser Ile Arg Gly Met Asp Lys Asn Arg Val Ser Leu Thr Val
            100                 105                 110

Asp Gly Val Ser Gln Ile Gln Ser Tyr Thr Ala Gln Ala Ala Leu Gly
        115                 120                 125

Gly Thr Arg Thr Ala Gly Ser Ser Gly Ala Ile Asn Glu Ile Glu Tyr
    130                 135                 140

Glu Asn Val Lys Ala Val Glu Ile Ser Lys Gly Ser Asn Ser Ser Glu
145                 150                 155                 160

Tyr Gly Asn Gly Ala Leu Ala Gly Ser Val Ala Phe Gln Thr Lys Thr
                165                 170                 175
```

```
Ala Ala Asp Ile Ile Gly Glu Gly Lys Gln Trp Gly Ile Gln Ser Lys
            180                 185                 190

Thr Ala Tyr Ser Gly Lys Asp His Ala Leu Thr Gln Ser Leu Ala Leu
        195                 200                 205

Ala Gly Arg Ser Gly Gly Ala Glu Ala Leu Leu Ile Tyr Thr Lys Arg
    210                 215                 220

Arg Gly Arg Glu Ile His Ala His Lys Asp Ala Gly Lys Gly Val Gln
225                 230                 235                 240

Ser Phe Asn Arg Leu Pro Ile Cys Arg Phe Gly Asn Asn Thr Tyr Thr
                245                 250                 255

Asp Cys Thr Pro Arg Asn Ile Gly Gly Asn Gly Tyr Tyr Ala Ala Val
            260                 265                 270

Gln Asp Asn Val Arg Leu Gly Arg Trp Ala Asp Val Gly Ala Gly Ile
        275                 280                 285

Arg Tyr Asp Tyr Arg Ser Thr His Ser Glu Asp Lys Ser Val Ser Thr
    290                 295                 300

Gly Thr His Arg Asn Leu Ser Trp Asn Ala Gly Val Val Leu Lys Pro
305                 310                 315                 320

Phe Thr Trp Met Asp Leu Thr Tyr Arg Ala Ser Thr Gly Phe Arg Leu
                325                 330                 335

Pro Ser Phe Ala Glu Met Tyr Gly Trp Arg Ala Gly Glu Ser Leu Lys
            340                 345                 350

Thr Leu Asp Leu Lys Pro Glu Lys Ser Phe Asn Arg Glu Ala Gly Ile
        355                 360                 365

Val Phe Lys Gly Asp Phe Gly Asn Leu Glu Ala Ser Tyr Phe Asn Asn
    370                 375                 380

Ala Tyr Arg Asp Leu Ile Ala Phe Gly Tyr Glu Thr Arg Thr Gln Asn
385                 390                 395                 400

Gly Gln Thr Ser Ala Ser Gly Asp Pro Gly Tyr Arg Asn Ala Gln Asn
                405                 410                 415

Ala Arg Ile Ala Gly Ile Asn Ile Leu Gly Lys Ile Asp Trp His Gly
            420                 425                 430

Val Trp Gly Gly Leu Pro Asp Gly Leu Tyr Ser Thr Leu Ala Tyr Asn
        435                 440                 445

Arg Ile Lys Val Lys Asp Ala Asp Arg Ala Asp Arg Thr Phe Val Thr
    450                 455                 460

Ser Tyr Leu Phe Asp Ala Val Gln Pro Ser Arg Tyr Val Leu Gly Leu
465                 470                 475                 480

Gly Tyr Asp His Pro Asp Gly Ile Trp Gly Ile Asn Thr Met Phe Thr
                485                 490                 495
```

```
Tyr Ser Lys Ala Lys Ser Val Asp Glu Leu Leu Gly Ser Gln Ala Leu
            500                 505                 510

Leu Asn Gly Asn Ala Asn Ala Lys Lys Ala Ala Ser Arg Arg Thr Arg
        515                 520                 525

Pro Trp Tyr Val Thr Asp Val Ser Gly Tyr Tyr Asn Ile Lys Lys His
    530                 535                 540

Leu Thr Leu Arg Ala Gly Val Tyr Asn Leu Leu Asn Tyr Arg Tyr Val
545                 550                 555                 560

Thr Trp Glu Asn Val Arg Gln Thr Ala Gly Gly Ala Val Asn Gln His
                565                 570                 575

Lys Asn Val Gly Val Tyr Asn Arg Tyr Ala Ala Pro Gly Arg Asn Tyr
            580                 585                 590

Thr Phe Ser Leu Glu Met Lys Phe
        595                 600
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 607 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Asn Lys Lys His Gly Phe Gln Leu Thr Leu Thr Ala Leu Ala Val
  1               5                  10                  15

Ala Ala Ala Phe Pro Ser Tyr Ala Ala Asn Pro Glu Thr Ala Ala Pro
             20                  25                  30

Asp Ala Ala Gln Thr Gln Ser Leu Lys Glu Val Thr Val Arg Ala Ala
         35                  40                  45

Lys Val Gly Arg Arg Ser Lys Glu Ala Thr Gly Leu Gly Lys Ile Ala
     50                  55                  60

Lys Thr Ser Glu Thr Leu Asn Lys Glu Gln Val Leu Gly Ile Arg Asp
 65                  70                  75                  80

Leu Thr Arg Tyr Asp Pro Gly Val Ala Val Val Glu Gln Gly Asn Gly
                 85                  90                  95

Ala Ser Gly Glu Tyr Ser Ile Arg Gly Val Asp Lys Asn Arg Val Ala
            100                 105                 110

Val Ser Val Asp Gly Val Ala Gln Ile Gln Ala Phe Thr Val Gln Gly
        115                 120                 125

Ser Leu Ser Gly Tyr Gly Gly Arg Gly Gly Ser Gly Ala Ile Asn Glu
```

130 135 140

Ile Glu Tyr Glu Asn Ile Ser Thr Val Glu Ile Asp Lys Gly Ala Gly
145 150 155 160

Ser Ser Asp His Gly Ser Gly Ala Leu Gly Gly Ala Val Ala Phe Arg
165 170 175

Thr Lys Glu Ala Ala Asp Leu Ile Ser Asp Gly Lys Ser Trp Gly Ile
180 185 190

Gln Ala Lys Thr Ala Tyr Gly Ser Lys Asn Arg Gln Phe Met Lys Ser
195 200 205

Leu Gly Ala Gly Phe Ser Lys Asp Gly Trp Glu Gly Leu Leu Ile Arg
210 215 220

Thr Glu Arg Gln Gly Arg Glu Thr His Pro His Gly Asp Ile Ala Asp
225 230 235 240

Gly Val Ala Tyr Gly Ile Asn Arg Leu Ser Val Cys Gly Tyr Ile Glu
245 250 255

Thr Leu Arg Ser Arg Lys Cys Val Pro Arg Lys Ile Asn Gly Ser Asn
260 265 270

Ile His Ile Ser Leu Asn Asp Arg Phe Ser Ile Gly Lys Tyr Phe Asp
275 280 285

Phe Ser Leu Gly Gly Arg Tyr Asp Arg Lys Asn Phe Thr Thr Ser Glu
290 295 300

Glu Leu Val Arg Ser Gly Arg Tyr Val Asp Arg Ser Trp Asn Ser Gly
305 310 315 320

Ile Val Phe Lys Pro Asn Arg His Phe Ser Leu Ser Tyr Arg Ala Ser
325 330 335

Ser Gly Phe Arg Thr Pro Ser Phe Gln Glu Leu Phe Gly Ile Asp Ile
340 345 350

Tyr His Asp Tyr Pro Lys Gly Trp Gln Arg Pro Ala Leu Lys Ser Glu
355 360 365

Lys Ala Ala Asn Arg Glu Ile Gly Leu Gln Trp Lys Gly Asp Phe Gly
370 375 380

Phe Leu Glu Ile Ser Ser Phe Arg Asn Arg Tyr Thr Asp Met Ile Ala
385 390 395 400

Val Ala Asp His Lys Thr Lys Leu Pro Asn Gln Ala Gly Gln Leu Thr
405 410 415

Glu Ile Asp Ile Arg Asp Tyr Tyr Asn Ala Gln Asn Met Ser Leu Gln
420 425 430

Gly Val Asn Ile Leu Gly Lys Ile Asp Trp Asn Gly Val tyr Gly Lys
435 440 445

Leu	Pro	Glu	Gly	Leu	Tyr	Thr	Thr	Leu	Ala	Tyr	Asn	Arg	Ile	Lys	Pro
	450					455					460				
Lys	Ser	Val	Ser	Asn	Arg	Pro	Gly	Leu	Ser	Leu	Arg	Ser	Tyr	Ala	Leu
465					470					475					480
Asp	Ala	Val	Gln	Pro	Ser	Arg	Tyr	Val	Leu	Gly	Phe	Gly	Tyr	Asp	Gln
				485					490					495	
Pro	Glu	Gly	Lys	Trp	Gly	Ala	Asn	Ile	Met	Leu	Thr	Tyr	Ser	Lys	Gly
			500					505					510		
Lys	Asn	Pro	Asp	Glu	Leu	Ala	Tyr	Leu	Ala	Gly	Asp	Gln	Lys	Arg	Tyr
		515					520					525			
Ser	Thr	Lys	Arg	Ala	Ser	Ser	Ser	Trp	Ser	Thr	Ala	Asp	Val	Ser	Ala
	530					535					540				
Tyr	Leu	Asn	Leu	Lys	Lys	Arg	Leu	Thr	Leu	Arg	Ala	Ala	Ile	Tyr	Asn
545					550					555					560
Ile	Gly	Asn	Tyr	Arg	Tyr	Val	Thr	Trp	Glu	Ser	Leu	Arg	Gln	Thr	Ala
				565					570					575	
Glu	Ser	Thr	Ala	Asn	Arg	His	Gly	Gly	Asp	Ser	Asn	Tyr	Gly	Arg	Tyr
			580					585					590		
Ala	Ala	Pro	Gly	Arg	Asn	Phe	Ser	Leu	Ala	Leu	Gly	Met	Lys	Phe	
		595					600					605			

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAACAGGTCT CGGCATAG 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCGAATTCA AACAGGTCTC GGCATAG 27

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCGAATTCA AAAACTTCCA TTCCAGCGAT ACG 33

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TAAAACTTCC ATTCCAGCGA TACG 24

What is claimed is:

1. An isolated and purified recombinant nucleic acid encoding a hemoglobin receptor protein having an amino acid sequence that is the amino acid sequence depicted as Seq. I.D. No. 6.

2. A recombinant expression construct comprising a nucleic acid that encodes a hemoglobin receptor protein from a *Neisseria* species having an amino acid sequence that is the amino acid sequence depicted as Seq. I.D. No. 6.

3. A transformed cell culture comprising the recombinant expression construct of Claim 2.

4. An isolated and purified recombinant nucleic acid encoding a hemoglobin receptor protein having an amino acid sequence that is the amino acid sequence depicted as Seq. I.D. No. 8.

5. A recombinant expression construct comprising a nucleic acid that encodes a hemoglobin receptor protein from a *Neisseria* species having an amino acid sequence that is the amino acid sequence depicted as Seq. I.D. No. 8.

6. A transformed cell culture comprising the recombinant expression construct of Claim 5.

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Chicago, Illinois 60606
(312) 913-0001

ABSTRACT OF THE DISCLOSURE

The present invention relates to novel bacterial hemoglobin receptor proteins and genes that encode such proteins. The invention is directed toward the isolation, characterization, diagnostic and therapeutic use of bacterial hemoglobin receptor proteins, nucleic acid encoding such proteins, recombinant expression constructs comprising such nucleic acids and cells transformed therewith, and antibodies and epitopes of such hemoglobin receptor proteins. The invention relates particularly to hemoglobin receptor proteins and genes encoding such proteins from *Neisseria* species, especially *N. meningitidis* and serotypes thereof, and *N. gonorrhoeae*. Methods for the diagnostic and therapeutic use of the proteins, epitopes, antibodies and nucleic acids of the invention are also provided, including the use of the proteins, epitopes, antibodies and nucleic acids of the invention for the production of vaccines effectinve in providing immunization of a human against infection by pathogenic bacteria of *Neisseria* species.

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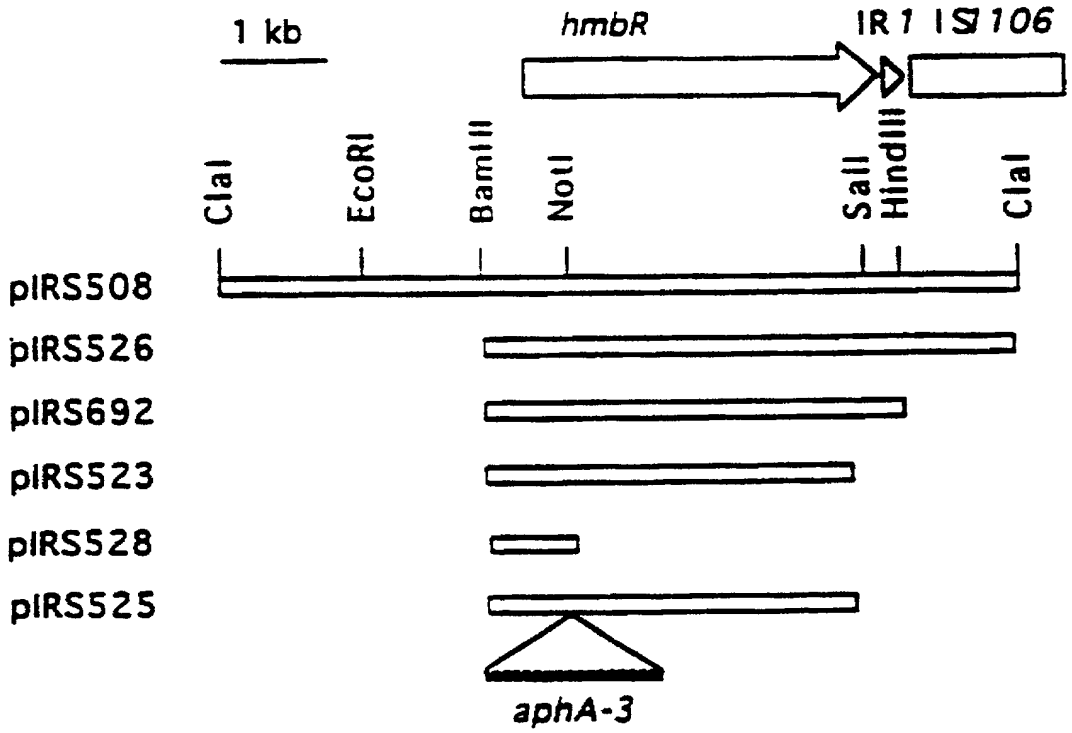
Ten South Wacker Drive, Suite 3000
Chicago, Illinois 60606
(312) 715-1000

Figure 1


1 kb
hmbR
IR
1
IS
106
ClaI
EcoRI
BamHI
NotI
SalI
HindIII
ClaI
pIRS508
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pIRS692
pIRS523
pIRS528
pIRS525
aphA-3

FIG. 2A

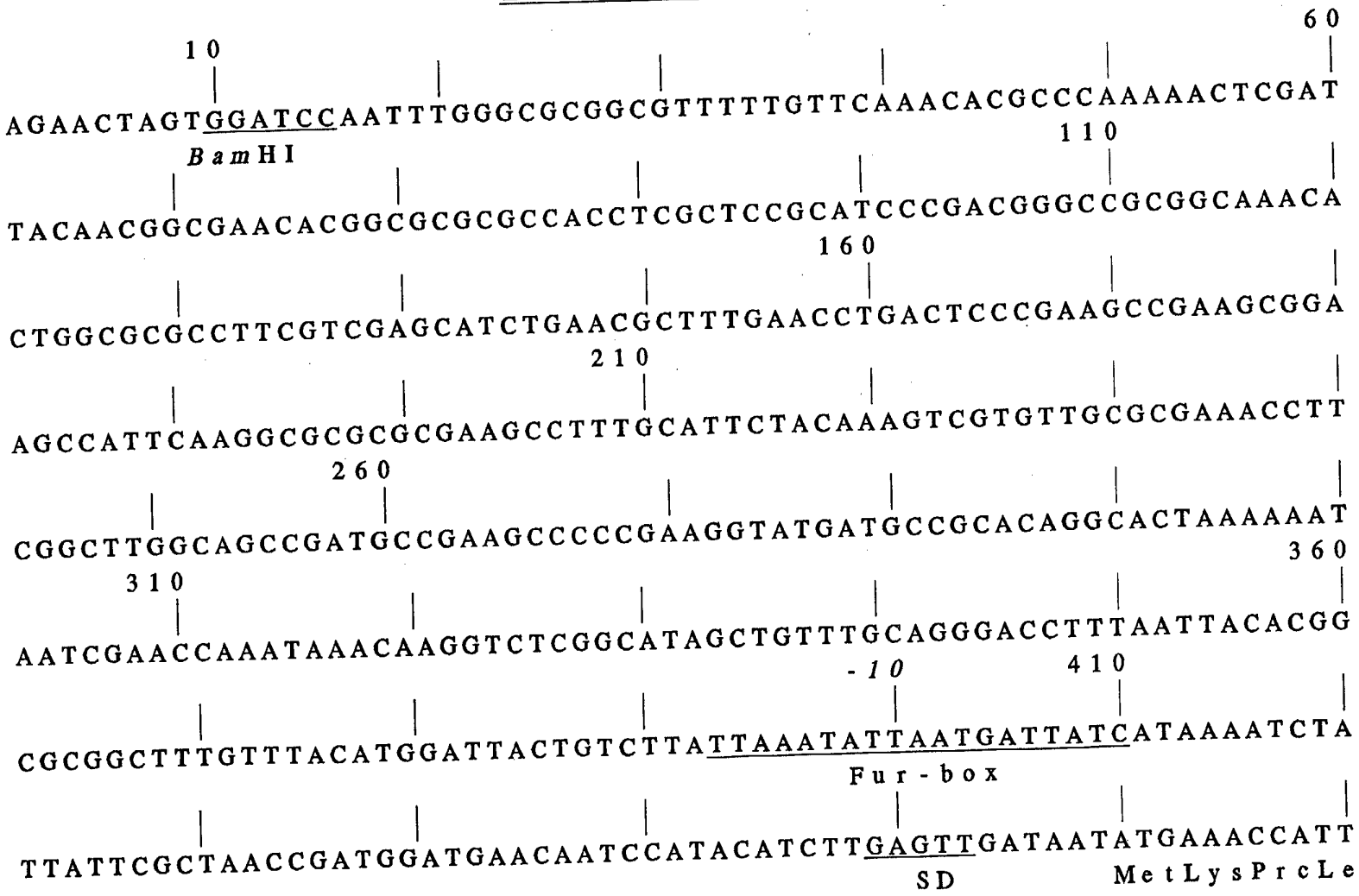

FIG. 2B

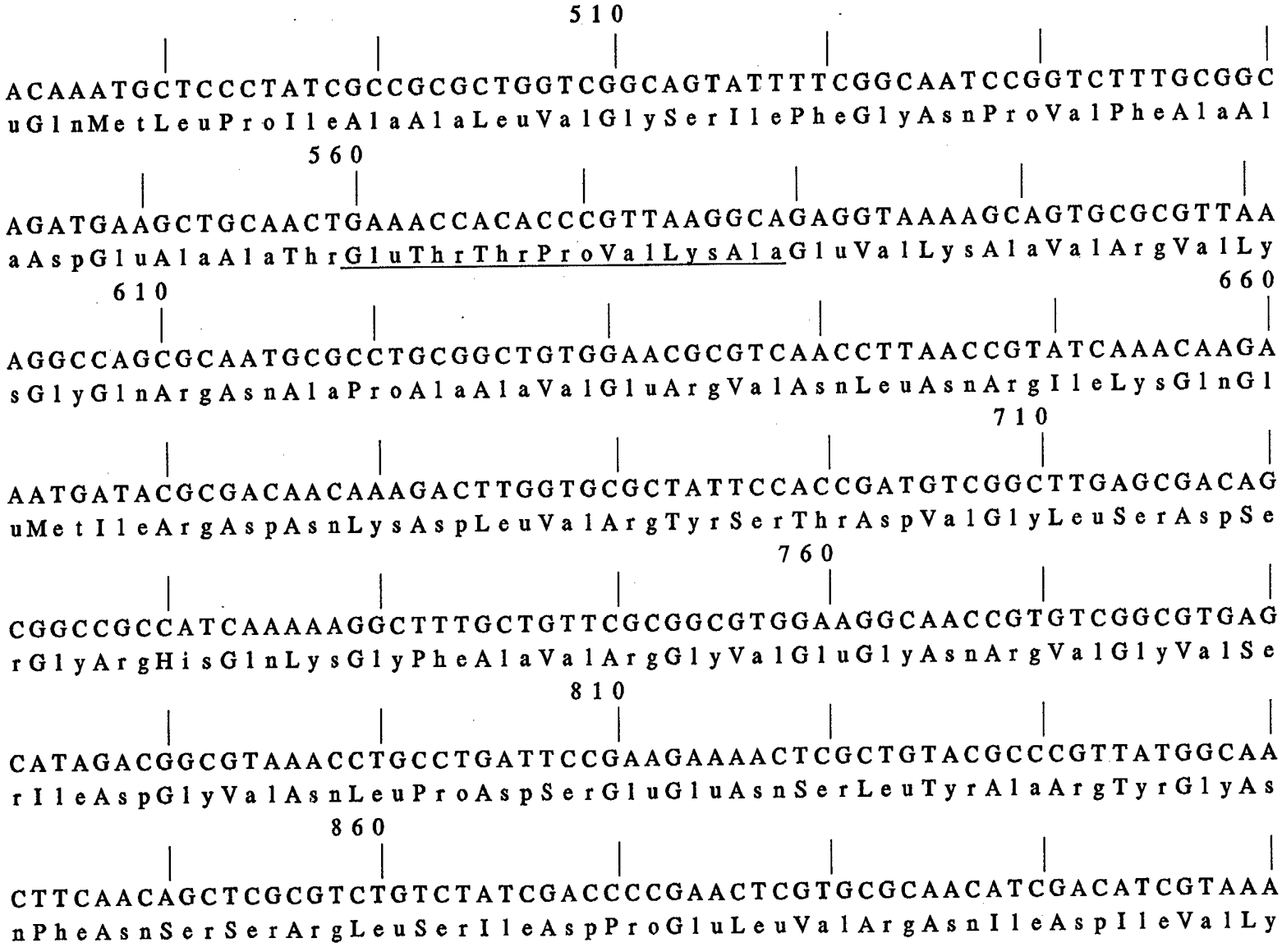
510
ACAAATGCTCCCTATCGCCGCGCTGGTCGGCAGTATTTTCGGCAATCCGGTCTTTGCGGC
uGlnMetLeuProIleAlaAlaLeuValGlySerIlePheGlyAsnProValPheAlaAl
560
AGATGAAGCTGCAACTGAAACCACACCCGTTAAGGCAGAGGTAAAAGCAGTGCGCGTTAA
aAspGluAlaAlaThrGluThrThrProValLysAlaGluValLysAlaValArgValLy
610
660
AGGCCAGCGCAATGCGCCTGCGGCTGTGGAACGCGTCAACCTTAACCGTATCAAACAAGA
sGlyGlnArgAsnAlaProAlaAlaValGluArgValAsnLeuAsnArgIleLysGlnGl
710
AATGATACGCGACAACAAAGACTTGGTGCGCTATTCCACCGATGTCGGCTTGAGCGACAG
uMetIleArgAspAsnLysAspLeuValArgTyrSerThrAspValGlyLeuSerAspSe
760
CGGCCGCCATCAAAAAGGCTTTGCTGTTCGCGGCGTGGAAGGCAACCGTGTCGGCGTGAG
rGlyArgHisGlnLysGlyPheAlaValArgGlyValGluGlyAsnArgValGlyValSe
810
CATAGACGGCGTAAACCTGCCTGATTCCGAAGAAAACTCGCTGTACGCCCGTTATGGCAA
rIleAspGlyValAsnLeuProAspSerGluGluAsnSerLeuTyrAlaArgTyrGlyAs
860
CTTCAACAGCTCGCGTCTGTCTATCGACCCCGAACTCGTGCGCAACATCGACATCGTAAA
nPheAsnSerSerArgLeuSerIleAspProGluLeuValArgAsnIleAspIleValLy

FIG. 2C


910
960
AGGGGCGGACTCTTTCAATACCGGCAGCGGCGCCTTGGGCGGCGGTGTGAATTACCAAAC
sGlyAlaAspSerPheAsnThrGlySerGlyAlaLeuGlyGlyGlyValAsnTyrGlnTh
1010
CCTGCAAGGACGTGACTTACTGTTGCCTGAACGGCAGTTCGGCGTGATGATGAAAAACGG
rLeuGlnGlyArgAspLeuLeuLeuProGluArgGlnPheGlyValMetMetLysAsnGl
1060
TTACAGCACGCGTAACCGTGAATGGACAAATACCCTCGGTTTCGGCGTGAGCAACGACCG
yTyrSerThrArgAsnArgGluTrpThrAsnThrLeuGlyPheGlyValSerAsnAspAr
1110
CGTGGATGCCGCTTTGCTGTATTCGCAACGGCGCGGCCATGAAACTGAAAGCGCGGGCAA
gValAspAlaAlaLeuLeuTyrSerGlnArgArgGlyHisGluThrGluSerAlaGlyLy
1160
GCGTGGTTATCCGGTAGAGGGTGCTGGTAGCGGAGCGAATATCCGTGGTTCTGCGCGCGG
sArgGlyTyrProValGluGlyAlaGlySerGlyAlaAsnIleArgGlySerAlaArgGl
1210
1260
TATTCCTGATCCGTCCCAACACAAATACCACAGCTTCTTGGGTAAGATTGCTTATCAAAT
yIleProAspProSerGlnHisLysTyrHisSerPheLeuGlyLysIleAlaTyrGlnIl
1310
CAACGACAACCACCGCATCGGCGCATCGCTCAACGGTCAGCAGGGGCATAATTACACGGT
eAsnAspAsnHisArgIleGlyAlaSerLeuAsnGlyGlnGlnGlyHisAsnTyrThrVa

FIG. 2D

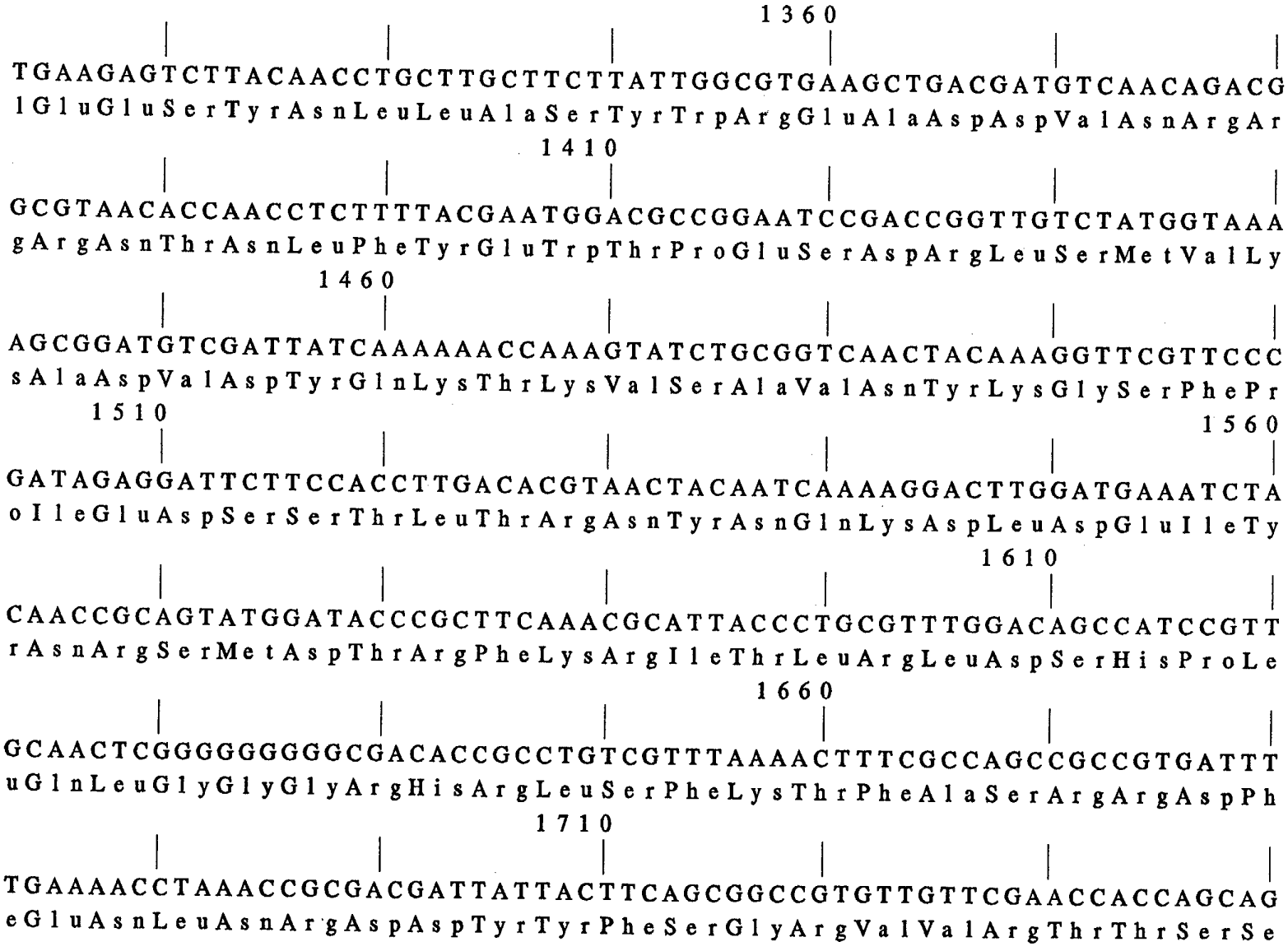

FIG. 2E

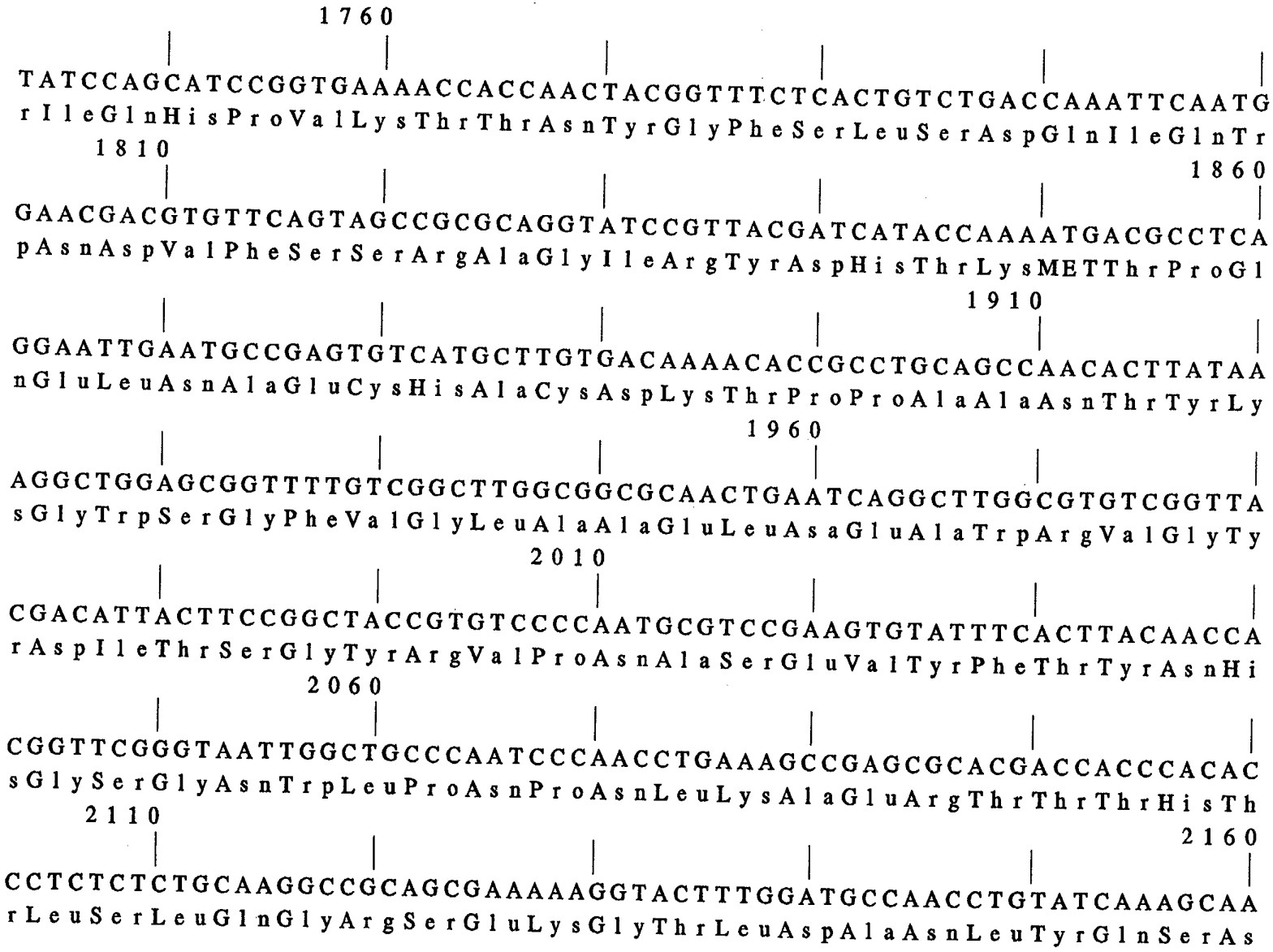
1760
TATCCAGCATCCGGTGAAAACCACCAACTACGGTTTCTCACTGTCTGACCAAATTCAATG
rIleGlnHisProValLysThrThrAsnTyrGlyPheSerLeuSerAspGlnIleGlnTr
1810
1860
GAACGACGTGTTCAGTAGCCGCGCAGGTATCCGTTACGATCATACCAAAATGACGCCTCA
pAsnAspValPheSerSerArgAlaGlyIleArgTyrAspHisThrLysMETThrProGl
1910
GGAATTGAATGCCGAGTGTCATGCTTGTGACAAAACACCGCCTGCAGCCAACACTTATAA
nGluLeuAsnAlaGluCysHisAlaCysAspLysThrProProAlaAlaAsnThrTyrLy
1960
AGGCTGGAGCGGTTTTGTCGGCTTGGCGGCGCAACTGAATCAGGCTTGGCGTGTCGGTTA
sGlyTrpSerGlyPheValGlyLeuAlaAlaGluLeuAsaGluAlaTrpArgValGlyTy
2010
CGACATTACTTCCGGCTACCGTGTCCCCAATGCGTCCGAAGTGTATTTCACTTACAACCA
rAspIleThrSerGlyTyrArgValProAsnAlaSerGluValTyrPheThrTyrAsnHi
2060
CGGTTCGGGTAATTGGCTGCCCAATCCCAACCTGAAAGCCGAGCGCACGACCACCCACAC
sGlySerGlyAsnTrpLeuProAsnProAsnLeuLysAlaGluArgThrThrThrHisTh
2110
2160
CCTCTCTCTGCAAGGCCGCAGCGAAAAAGGTACTTTGGATGCCAACCTGTATCAAAGCAA
rLeuSerLeuGlnGlyArgSerGluLysGlyThrLeuAspAlaAsnLeuTyrGlnSerAs

FIG. 2F

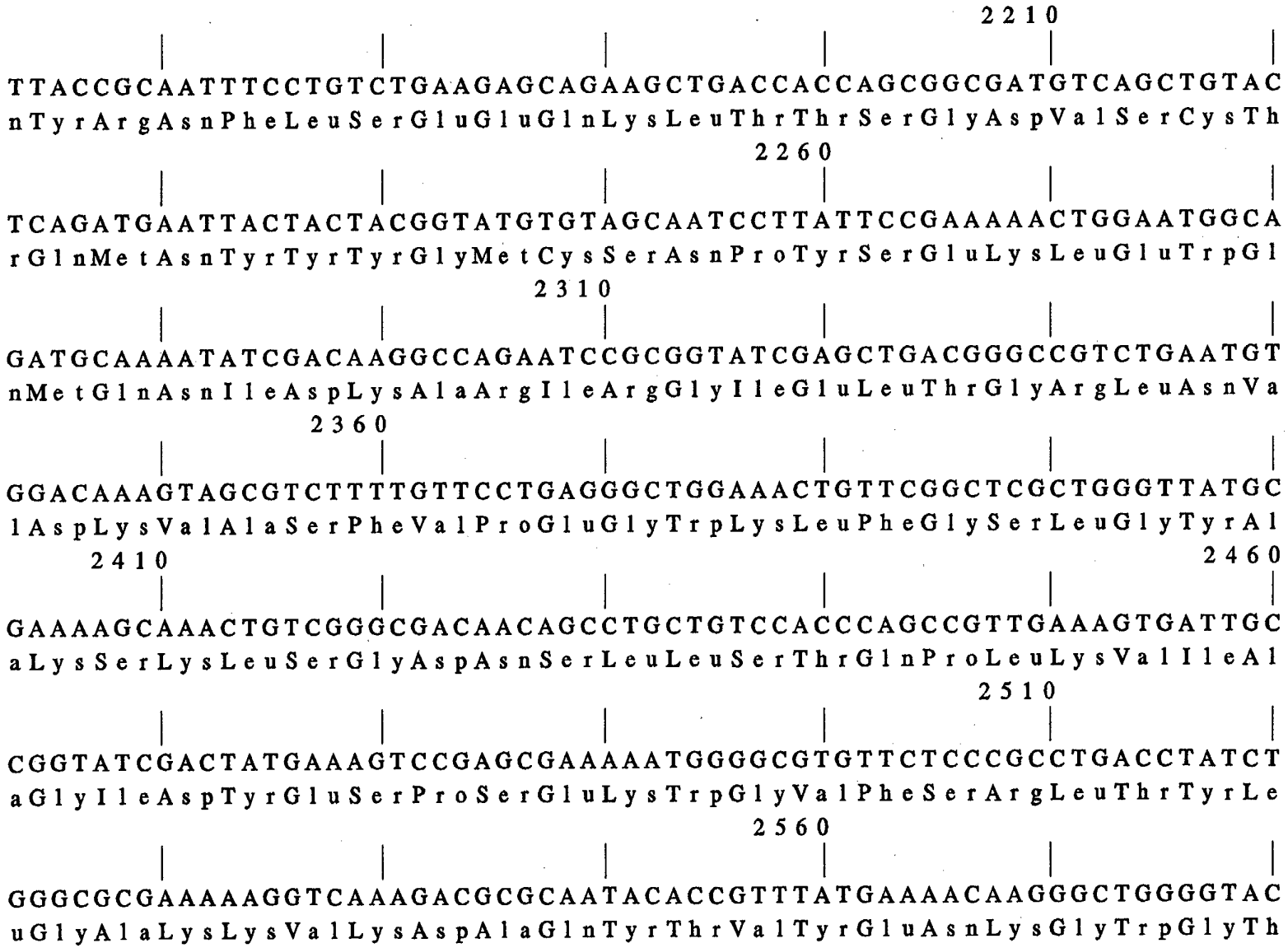
2210
TTACCGCAATTTCCTGTCTGAAGAGCAGAAGCTGACCACCAGCGGCGATGTCAGCTGTAC
nTyrArgAsnPheLeuSerGluGluGlnLysLeuThrThrSerGlyAspValSerCysTh
2260
TCAGATGAATTACTACTACGGTATGTGTAGCAATCCTTATTCCGAAAAACTGGAATGGCA
rGlnMetAsnTyrTyrTyrGlyMetCysSerAsnProTyrSerGluLysLeuGluTrpGl
2310
GATGCAAAATATCGACAAGGCCAGAATCCGCGGTATCGAGCTGACGGGCCGTCTGAATGT
nMetGlnAsnIleAspLysAlaArgIleArgGlyIleGluLeuThrGlyArgLeuAsnVa
2360
GGACAAAGTAGCGTCTTTTGTTCCTGAGGGCTGGAAACTGTTCGGCTCGCTGGGTTATGC
lAspLysValAlaSerPheValProGluGlyTrpLysLeuPheGlySerLeuGlyTyrAl
2410
2460
GAAAAGCAAACTGTCGGGCGACAACAGCCTGCTGTCCACCCAGCCGTTGAAAGTGATTGC
aLysSerLysLeuSerGlyAspAsnSerLeuLeuSerThrGlnProLeuLysValIleAl
2510
CGGTATCGACTATGAAAGTCCGAGCGAAAAATGGGGCGTGTTCTCCCGCCTGACCTATCT
aGlyIleAspTyrGluSerProSerGluLysTrpGlyValPheSerArgLeuThrTyrLe
2560
GGGCGCGAAAAAGGTCAAAGACGCGCAATACACCGTTTATGAAAACAAGGGCTGGGGTAC
uGlyAlaLysLysValLysAspAlaGlnTyrThrValTyrGluAsnLysGlyTrpGlyTh

FIG. 2G

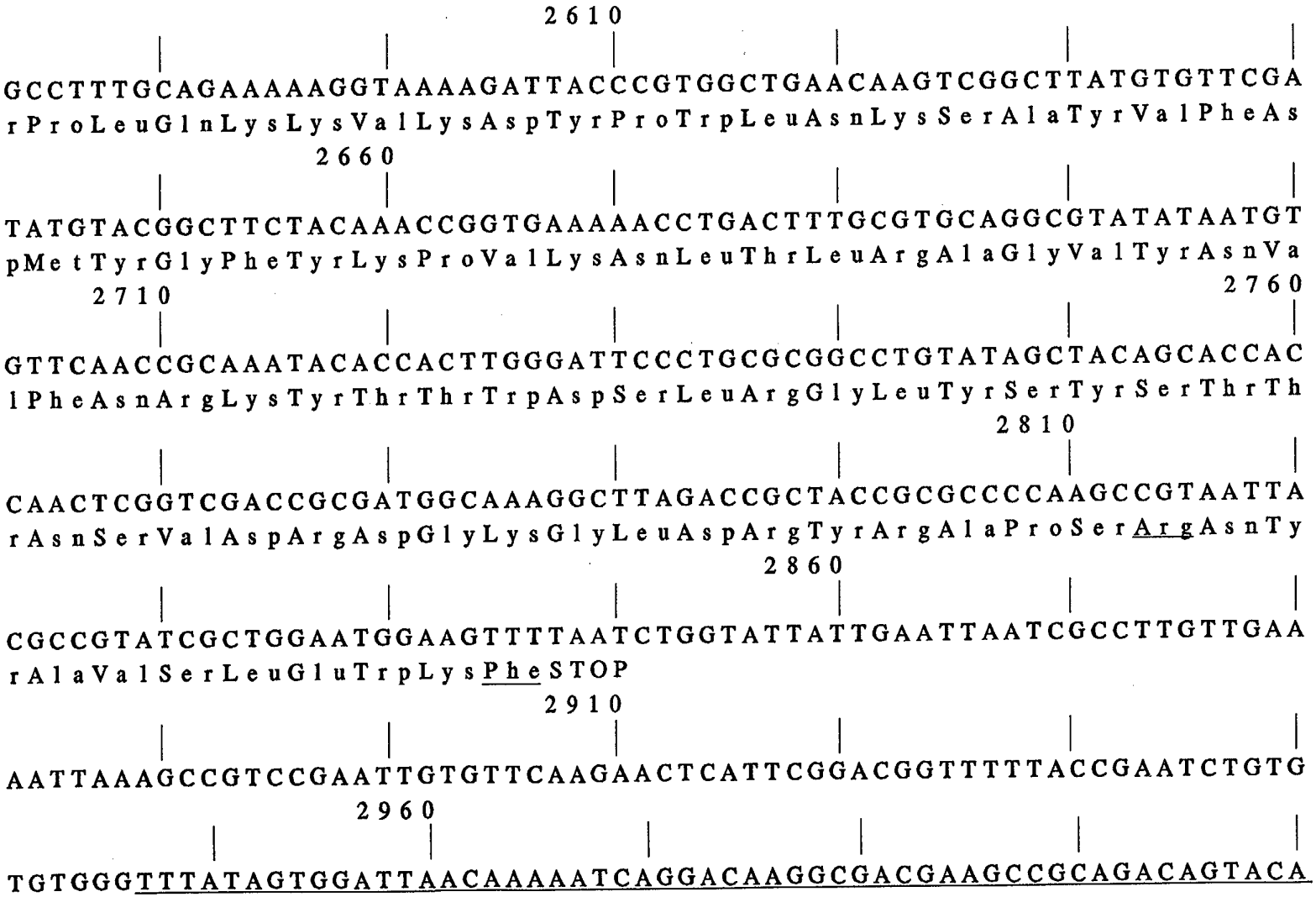

FIG. 2H

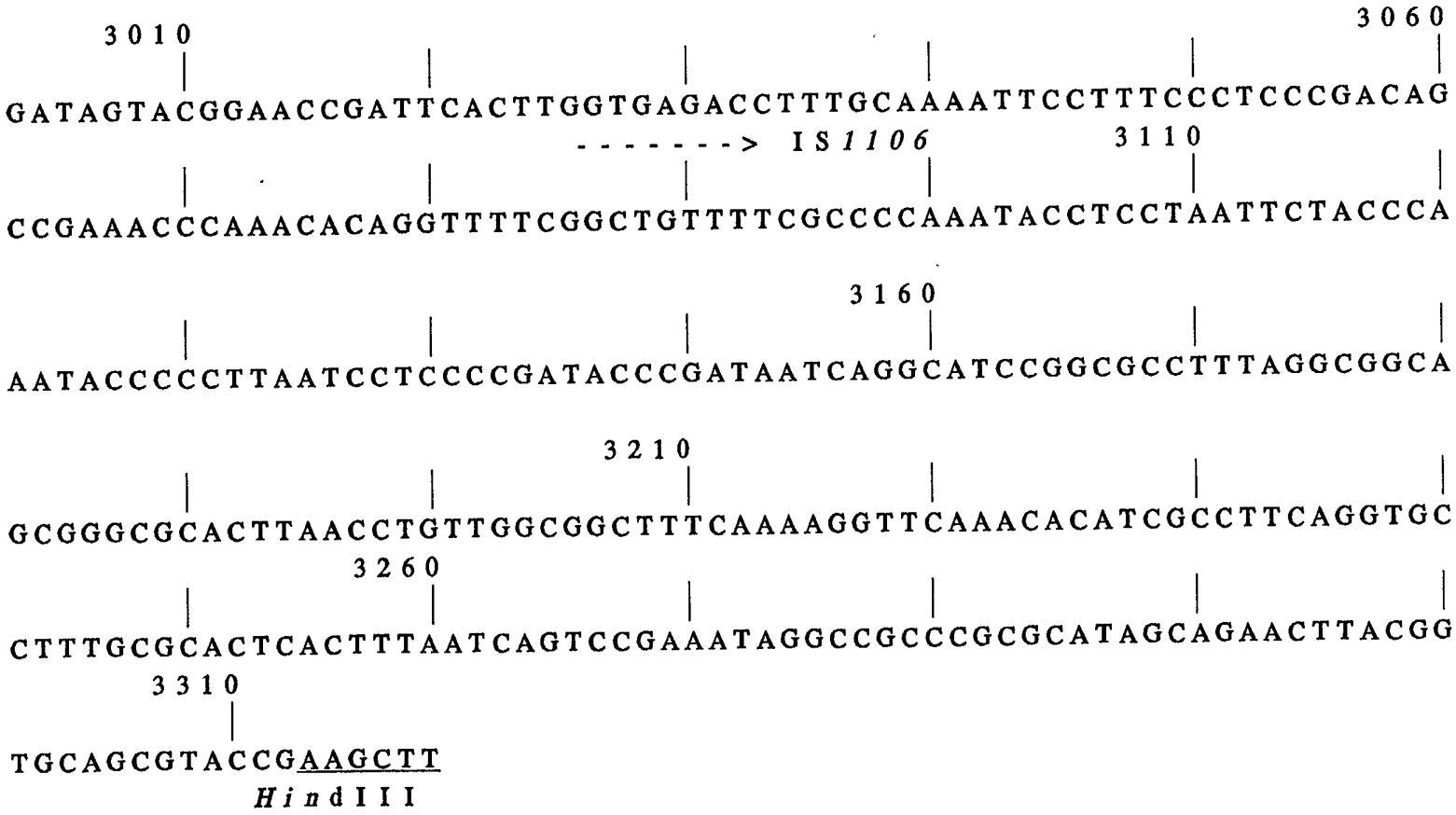

Figure 3

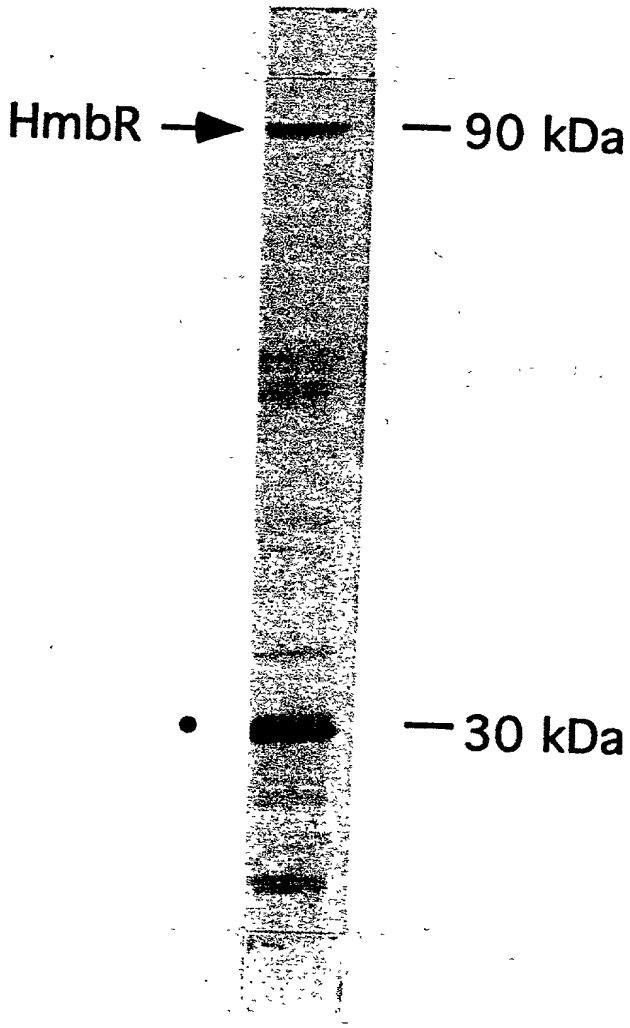

FIG. 4A


```
TBP1M   MQQQHLFRLNILCLSLMTALPVYA---ENVQAEQAQEKQLDTIOVKAKKQ   47
LBPA    MNKKHGFQLTLTALAVAAAFPSYAANPETAAPDAAQTQSLKEVTVRAAKV   50
HMBR    MKPLQMLPIAALVGSIFGN-PVFAADEAATETTPVKAE------VKAVR    43
        *.  . ....  .. .. * .*  ..........  *.* .

TBP1M   KTRRDNEVTGLGKLVKSSDTLSKEQVLNIRDLTRYDPGIAVVEQGRGASS   97
LBPA    -GRRSKEATGLGKIAKTSETLNKEQVLGIRDLTRYDPGVAVVEQGNGASG   99
HMBR    KGQRNA-PAAVERV--NLNRIKQEMIRDNKDLVRYSTDVGLSDSGRHQK-   89
          ..*.  ..... . . ...* . . .**.**..... . *. ..

TBP1M   GYSIRGMDKNRVSLTVDGVSQIQSYTAQAALGGTRTAGSSGAINEIEYEN  147
LBPA    GYSIRGVDKNRVAVSVDGVAQIQAFTVQGSLSGYGGRGGSGAINEIEYEN  149
HMBR    GFAVRGVEGNRVGVSIDGVNLPDS--EENSLYARYGNFNSSRLS-IDPEL  136
        *...**.. ***...***. ..   ...* . . .*. .. *. *

TBP1M   VKAVEISKGSNSSEYGNGALAGSVAFQTKTAADIIGEGKQWGIQSKTAYS  197
LBPA    ISTVEIDKGAGSSDHGSGALGGAVAFRTKEAADLISDGKSWGIQAKTAYG  199
HMBR    VRNIDIVKGADSFNTGSGALGGGVYNQTLQGRDLLLPERQFGVMMKNGYS  186
        .....* **..*  . *.***.*.*...*   . *..  .. .*.  *..*.

TBP1M   GKDHALTQSLALAGRSGGAEALLIYTKRRGREIHAHKDAGKGVQ-SFNRL  246
LBPA    SKNRQFMKSLGAGFSKDGWEGLLIRTERQGRETHPHGDIADGVAYGINRL  249
HMBR    TRNREWTNTLGFGVSNDRVDAALLYSQRRGHETESAG------------  223
        .....  ..*. . ...  .. *. ..*.*.*...
```

FIG. 4B


```
TBP1M   PICRFGNNTYT-DCTPRNIGGNGYYAAVQDNVRLGRWADVGAGIRYDYRS   601
LBPA    SVCGYIETLRSRKCVPRKINGSNIHISLNDRFSIGKYFDFSLGGRYDRKN   635
HMBR    -----------SSIQHPVKTTNYGFSLSDQIQWNDVFSSRAGIRYDHTK   460
                   ..... . ...  .. *.  .  .  * *** .

TBP1M   THSED--------KSVSTGTHRNLSWNAGVVLKP--FTWMDLTYRASTGF   641
LBPA    FTTSE--------ELVRSGRYVDRSWNSGIVFKP--NRHFSLSYRASSGF   675
HMBR    MTPQELNAECHACDKTPPAANTYKGWSGFVGLAAQLNQAWRVGYDITSGY   510
          . .       . ....    .*. . . .      . .*  . .*.

TBP1M   RLPSFAEMYGWRA----GESLKTLDLKPEKSFNREAGIVFKGDFGNLEAS   687
LBPA    RTPSFQELFGIDIYHDYPKGWQRPALKSEKAANREIGLQWKGDFGFLEIS   725
HMBR    RVPNASEVY-FTYNHGSGNWLPNPNLKAERTTTHTLSLQGRSEKGTLDAN   559
        * *.  *..       .    .**.*.. ... .. ... * *. .

TBP1M   YFNNAYRDLIAFGYET---RTQNGQTSASGDPGYR---------------   719
LBPA    SFRNRYTDMIAVADHKTKLPNQAGQLTEIDIRDYY---------------   760
HMBR    LYQSNYRNFLS---EEQKLTT-SGDVSCTQMNYYYGMCSNPYSEKLEWQM   605
          ... * .... .. . .*. .       *

TBP1M   -NAQNARIAGINILGKIDWHGVWGGLPDG--LYSTLAYNRIKVKDADIRA   766
LBPA    -NAQNMSLQGVNILGKIDWNGVYGKLPEG--LYTTLAYNRIKPKSVSNRP   807
HMBR    QNIDKARIRGIELTGRLNVDKVASFVPEGWKLFGSLGYAKSKLSG-----   650
          * .. .. *... *... . * . .*.*  *...*.*.. * ..
```

FIG. 4C


```
TBP1M    DRTFVTSYLFDAVQPSRYVLGLGYDHPDGIWGINTMFTYSKAKSVDE---   813
LBPA     GLSL-RSYALDAVQPSRYVLGFGYDQPEGKWGANIMLTYSKGKNPDE---   853
HMBR     DNSLLST------QPLKVIAGIDYESPSEKWGVFSRLTYLGAKKVKDAQY   694
           .  ..   .        **  .  .  *..*.  *..  **    ..**  .*.  ..

TBP1M    -LLGSQALLNGNANAKKAASRRTRPWYVTDVSGYYNIKKHLTLRAGVYNL   862
LBPA     -L----AYLAGDQK-RYSTKRASSSWSTADVSAYLNLKKRLTLRAAIYNI   897
HMBR     TVYENKGWGTPLQKKVKDYPWLNKSAYVFDMYGFYKPVKNLTLRAGVYNV   744
           .    .   .  ..   .  .  ...  . *.  ..  .  *.*****..**.

TBP1M    LNYRYVTWENVRQ--TAGGAVNQHKNVGVYNRYAAPGRNYTFSLEMKF    908
LBPA     GNYRYVTWESLRQ--TAESTANRHGGDSNYGRYAAPGRNFSLALEMKF    943
HMBR     FNRKYTTWDSLRGLYSYSTTNSVDRDGKGLDRYRAPSRNYAVSLEWKF    792
           *  .*.*...*     .  ...  .  .  .    .**  **.**..  .**  **
```

Figure 5

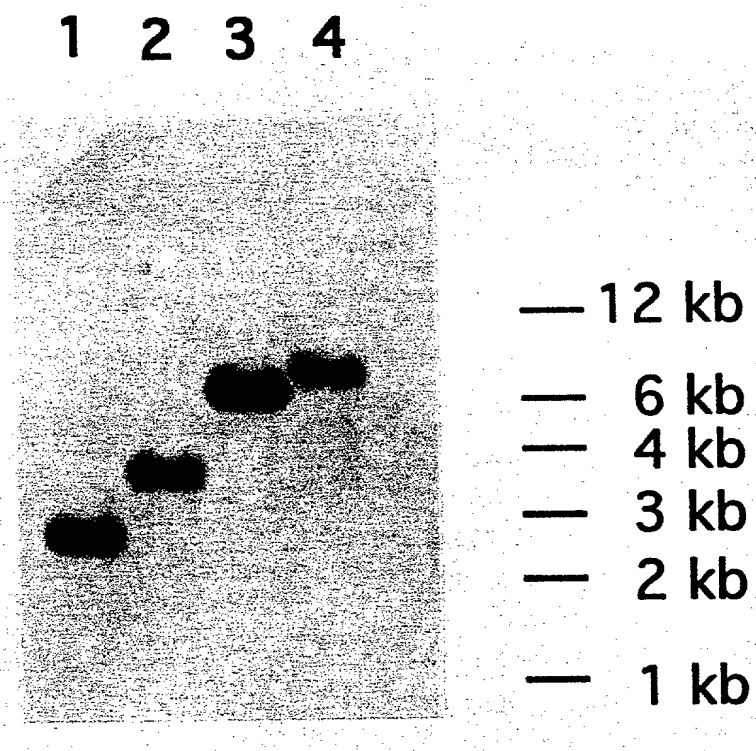

FIG. 6

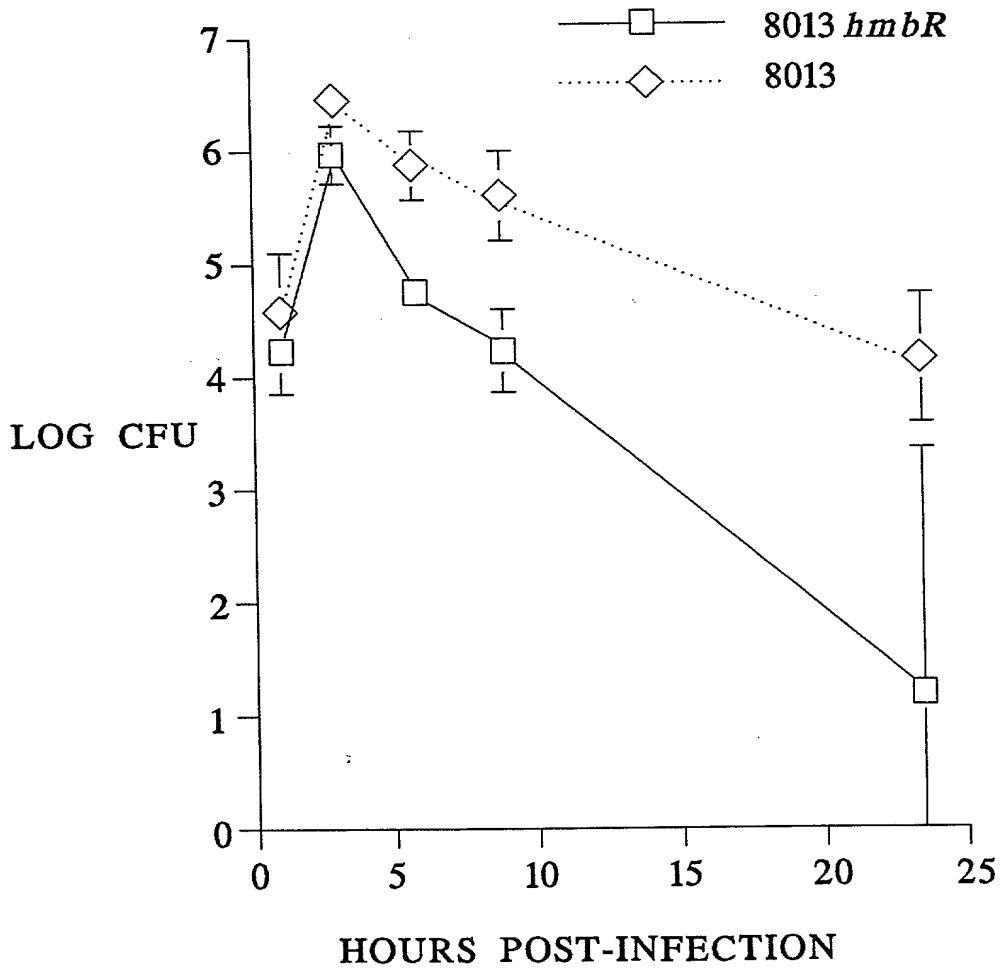
8013 hmbR
8013
LOG CFU
0
1
2
3
4
5
6
7
0
5
10
15
20
25
HOURS POST-INFECTION

FIG. 7A

```
ATG AAA CCA TTA CAA ATG CCC CCT ATC GCC GCG CTG CTC GGC AGT ATT      48
Met Lys Pro Leu Gln Met Pro Pro Ile Ala Ala Leu Leu Gly Ser Ile
 1               5                  10                  15

TTC GGC AAT CCG GTC TTT GCG GCA GAT GAA GCT GCA ACT GAA ACC ACA      96
Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
            20                  25                  30

CCC GTT AAG GCA GAG GTA AAA GCA GTG CGC GTT AAA GGT CAG CGC AAT     144
Pro Val Lys Ala Glu Val Lys Ala Val Arg Val Lys Gly Gln Arg Asn
        35                  40                  45

GCG CCT GCG GCT GTG GAA CGC GTC AAC CTT AAC CGT ATC AAA CAA GAA     192
Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
    50                  55                  60

ATG ATA CGC GAC AAT AAA GAC TTG GTG CGC TAT TCC ACC GAT GTC GGC     240
Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65                  70                  75                  80

TTG AGC GAC AGG AGC CGT CAT CAA AAA GGC TTT GCC ATT CGC GGC GTG     288
Leu Ser Asp Arg Ser Arg His Gln Lys Gly Phe Ala Ile Arg Gly Val
                85                  90                  95
```

FIG. 7B

```
GAA GGC GAC CGT GTC GGC GTT AGT ATT GAC GGC GTA AAC CTG CCT GAT      336
Glu Gly Asp Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
            100                 105                 110

TCC GAA GAA AAC TCG CTG TAC GCC CGT TAT GGC AAC TTC AAC AGC TCG      384
Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

CGT CTG TCT ATC GAC CCC GAA CTC GTG CGC AAC ATC GAC ATC GTA AAA      432
Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
    130                 135                 140

GGG GCG GAC TCT TTC AAT ACC GGC AGC GGC GCC TTG GGC GGC GGT GTG      480
Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

AAT TAC CAA ACC CTG CAA GGA CGT GAC TTA CTG TTG CCT GAA CGG CAG      528
Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
                165                 170                 175

TTC GGC GTG ATG ATG AAA AAC GGT TAC AGC ACG CGT AAC CGT GAA TGG      576
Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190
```

FIG. 7C

```
ACA AAT ACC CTC GGT TTC GGC GTG AGC AAC GAC CGC GTG GAT GCC GCT      624
Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

TTG CTG TAT TCG CAA CGG CGC GGC CAT GAA ACT GAA AGC GCG GGC AAG      672
Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
    210                 215                 220

CGT GGT TAT CCG GTA GAG GGT GCT GGT AGC GGA GCG AAT ATC CGT GGT      720
Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225                 230                 235                 240

TCT GCG CGC GGT ATT CCT GAT CCG TCC CAA CAC AAA TAC CAC AGC TTC      768
Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
                245                 250                 255

TTG GGT AAG ATT GCT TAT CAA ATC AAC GAC AAC CAC CGC ATC GGC GCA      816
Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
            260                 265                 270

TCG CTC AAC GGT CAG CAG GGG CAT AAT TAC ACG GTT GAA GAG TCT TAC      864
Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
        275                 280                 285
```

FIG. 7D

```
AAC CTG CTT GCT TCT TAT TGG CGT GAA GCT GAC GAT GTC AAC AGA CGG      912
Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
    290                 295                 300

CGT AAC ACC AAC CTC TTT TAC GAA TGG ACG CCG GAA TCC GAC CGG TTG      960
Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305                 310                 315                 320

TCT ATG GTA AAA GCG GAT GTC GAT TAT CAA AAA ACC AAA GTA TCT GCG     1008
Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
                325                 330                 335

GTC AAC TAC AAA GGT TCG TTC CCG ACG AAT TAC ACC ACA TGG GAA ACC     1056
Val Asn Tyr Lys Gly Ser Phe Pro Thr Asn Tyr Thr Thr Trp Glu Thr
            340                 345                 350

GAG TAC CAT AAA AAG GAA GTT GGC GAA ATC TAT AAC CGC AGC ATG GAT     1104
Glu Tyr His Lys Lys Glu Val Gly Glu Ile Tyr Asn Arg Ser Met Asp
        355                 360                 365

ACA ACC TTC AAA CGT ATT ACG CTG CGT ATG GAC AGC CAT CCG TTG CAA     1152
Thr Thr Phe Lys Arg Ile Thr Leu Arg Met Asp Ser His Pro Leu Gln
    370                 375                 380
```

FIG. 7E

```
CTC GGG GGG GGG CGA CAC CGC CTG TCG TTC AAA ACC TTT GCC GGG CAG      1200
Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Gly Gln
385                 390                 395                 400

CGT GAT TTT GAA AAC TTA AAC CGC GAC GAT TAC TAC TTC AGC GGC CGT      1248
Arg Asp Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Gly Arg
                405                 410                 415

GTT GTT CGA ACC ACC AAC AGT ATC CAG CAT CCG GTG AAA ACC ACC AAC      1296
Val Val Arg Thr Thr Asn Ser Ile Gln His Pro Val Lys Thr Thr Asn
            420                 425                 430

TAC GGT TTC TCG CTG TCC GAC CAA ATC CAA TGG AAC GAC GTG TTC AGT      1344
Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser
        435                 440                 445

AGC CGC GCA GGT ATC CGT TAC GAC CAC ACC AAA ATG ACG CCT CAG GAA      1392
Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu
    450                 455                 460

TTG AAT GCC GAC TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC AAC      1440
Leu Asn Ala Asp Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn
465                 470                 475                 480
```

FIG. 7F

```
ACT TAT AAA GGC TGG AGC GGA TTT GTC GGC TTG GCG GCG CAG CTG AGC      1488
Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Ser
                485                 490                 495

CAA ACA TGG CGT TTG GGT TAC GAT GTG ACC TCA GGT TTC CGC GTG CCG      1536
Gln Thr Trp Arg Leu Gly Tyr Asp Val Thr Ser Gly Phe Arg Val Pro
            500                 505                 510

AAT GCG TCT GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGC ACT TGG      1584
Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Thr Trp
        515                 520                 525

AAG CCT AAT CCT AAT TTG AAG GCA GAA CGC AGC ACC ACC CAC ACC CTG      1632
Lys Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr Leu
    530                 535                 540

TCC TTG CAG GGG CGC GGC GAC AAA GGG ACA CTG GAT GCC AAC CTG TAT      1680
Ser Leu Gln Gly Arg Gly Asp Lys Gly Thr Leu Asp Ala Asn Leu Tyr
545                 550                 555                 560

CAA AGC AAT TAC CGA AAC TTC CTG TCG GAA GAG CAG AAT CTG ACT GTC      1728
Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Asn Leu Thr Val
                565                 570                 575
```

FIG. 7G

```
AGC GGC ACA CCC GGC TGT ACT GAG GAG GAT GCT TAC TAC TAT AGA TGC   1776
Ser Gly Thr Pro Gly Cys Thr Glu Glu Asp Ala Tyr Tyr Tyr Arg Cys
            580                 585                 590

AGC GAC CCC TAC AAA GAA AAA CTG GAT TGG CAG ATG AAA AAT ATC GAC   1824
Ser Asp Pro Tyr Lys Glu Lys Leu Asp Trp Gln Met Lys Asn Ile Asp
        595                 600                 605

AAG GCC AGA ATC CGC GGT ATC GAG TTG ACA GGC CGT CTG AAT GTG GAC   1872
Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val Asp
    610                 615                 620

AAA GTA GCG TCT TTT GTT CCT GAG GGT TGG AAA CTG TTC GGC TCG CTG   1920
Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu
625                 630                 635                 640

GGT TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TCC ACA   1968
Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser Thr
            645                 650                 655

CAG CCG CTG AAA GTG ATT GCC GGT ATC GAC TAT GAA AGT CCG AGC GAA   2016
Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser Glu
            660                 665                 670
```

FIG. 7H

```
AAA TGG GGC GTA TTC TCC CGC CTG ACC TAT CTA GGC GCG AAA AAG GTC      2064
Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Val
        675                 680                 685

AAA GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC TGG GGT ACG CCT      2112
Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr Pro
    690                 695                 700

TTG CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT TAT      2160
Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr
705                 710                 715                 720

GTG TTT GAT ATG TAC GGC TTC TAC AAA CCG GCT AAA AAC CTG ACT TTG      2208
Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Ala Lys Asn Leu Thr Leu
                725                 730                 735

CGT GCA GGC GTG TAC AAC CTG TTC AAC CGC AAA TAC ACC ACT TGG GAT      2256
Arg Ala Gly Val Tyr Asn Leu Phe Asn Arg Lys Tyr Thr Thr Trp Asp
            740                 745                 750

TCC CTG CGC GGT TTA TAT AGC TAC AGC ACC ACC AAT GCG GTC GAC CGC      2204
Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ala val Asp Arg
        755                 760                 765
```

FIG. 7I

```
GAT GGC AAA GGC TTA GAC CGC TAC CGC GCC CCA GGC CGC AAT TAC GCC      2352
Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Gly Arg Asn Tyr Ala
    770                 775                 780

GTA TCG CTG GAA TGG AAG TTT TAA                                    2375
Val Ser Leu Glu Trp Lys Phe  *
785                 790
```

FIG. 8A

```
ATG AAA CCA TTA CAA ATG CTC CCT ATC GCC GCG CTG GTC GGC AGT ATT      48
Met Lys Pro Leu Gln Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
 1               5                  10                  15

TTC GGC AAT CCG GTC TTT GCG GCA GAT GAA GCT GCA ACT GAA ACC ACA      96
Phe Gly Asn Pro Val Phe Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
            20                  25                  30

CCC GTT AAG GCA GAG GTA AAA GCA GTG CGC GTT AAA GGC CAG CGC AAT     144
Pro Val Lys Ala Glu Val Lys Ala Val Arg Val Lys Gly Gln Arg Asn
        35                  40                  45

GCG CCT GCG GCT GTG GAA CGC GTC AAC CTT AAC CGT ATC AAA CAA GAA     192
Ala Pro Ala Ala Val Glu Arg Val Asn Leu Asn Arg Ile Lys Gln Glu
    50                  55                  60

ATG ATA CGC GAC AAC AAA GAC TTG GTG CGC TAT TCC ACC GAT GTC GGC     240
Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65                  70                  75                  80

TTG AGC GAC AGC GGC CGC CAT CAA AAA GGC TTT GCT GTT CGC GGC GTG     288
Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
            85                  90                  95
```

FIG. 8B

```
GAA GGC AAC CGT GTC GGC GTG AGC ATA GAC GGC GTA AAC CTG CCT GAT      336
Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Asn Leu Pro Asp
            100                 105                 110

TCC GAA GAA AAC TCG CTG TAC GCC CGT TAT GGC AAC TTC AAC AGC TCG      384
Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

CGT CTG TCT ATC GAC CCC GAA CTC GTG CGC AAC ATC GAC ATC GTA AAA      432
Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Asp Ile Val Lys
    130                 135                 140

GGG GCG GAC TCT TTC AAT ACC GGC AGC GGC GCC TTG GGC GGC GGT GTG      480
Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

AAT TAC CAA ACC CTG CAA GGA CGT GAC TTA CTG TTG CCT GAA CGG CAG      528
Asn Tyr Gln Thr Leu Gln Gly Arg Asp Leu Leu Leu Pro Glu Arg Gln
                165                 170                 175

TTC GGC GTG ATG ATG AAA AAC GGT TAC AGC ACG CGT AAC CGT GAA TGG      576
Phe Gly Val Met Met Lys Asn Gly Tyr Ser Thr Arg Asn Arg Glu Trp
            180                 185                 190
```

FIG. 8C

```
ACA AAT ACC CTC GGT TTC GGC GTG AGC AAC GAC CGC GTG GAT GCC GCT      624
Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

TTG CTG TAT TCG CAA CGG CGC GGC CAT GAA ACT GAA AGC GCG GGC AAG      672
Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Lys
    210                 215                 220

CGT GGT TAT CCG GTA GAG GGT GCT GGT AGC GGA GCG AAT ATC CGT GGT      720
Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Asn Ile Arg Gly
225                 230                 235                 240

TCT GCG CGC GGT ATT CCT GAT CCG TCC CAA CAC AAA TAC CAC AGC TTC      768
Ser Ala Arg Gly Ile Pro Asp Pro Ser Gln His Lys Tyr His Ser Phe
                245                 250                 255

TTG GGT AAG ATT GCT TAT CAA ATC AAC GAC AAC CAC CGC ATC GGC GCA      816
Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Asn His Arg Ile Gly Ala
            260                 265                 270

TCG CTC AAC GGT CAG CAG GGG CAT AAT TAC ACG GTT GAA GAG TCT TAC      864
Ser Leu Asn Gly Gln Gln Gly His Asn Tyr Thr Val Glu Glu Ser Tyr
        275                 280                 285
```

FIG. 8D

```
AAC CTG CTT GCT TCT TAT TGG CGT GAA GCT GAC GAT GTC AAC AGA CGG   911
Asn Leu Leu Ala Ser Tyr Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
    290                 295                 300

CGT AAC ACC AAC CTC TTT TAC GAA TGG ACG CCG GAA TCC GAC CGG TTG   960
Arg Asn Thr Asn Leu Phe Tyr Glu Trp Thr Pro Glu Ser Asp Arg Leu
305                 310                 315                 320

TCT ATG GTA AAA GCG GAT GTC GAT TAT CAA AAA ACC AAA GTA TCT GCG  1008
Ser Met Val Lys Ala Asp Val Asp Tyr Gln Lys Thr Lys Val Ser Ala
                325                 330                 335

GTC AAC TAC AAA GGT TCG TTC CCG ATA GAG GAT TCT TCC ACC TTG ACA  1056
Val Asn Tyr Lys Gly Ser Phe Pro Ile Glu Asp Ser Ser Thr Leu Thr
            340                 345                 350

CGT AAC TAC AAT CAA AAG GAC TTG GAT GAA ATC TAC AAC CGC AGT ATG  1104
Arg Asn Tyr Asn Gln Lys Asp Leu Asp Glu Ile Tyr Asn Arg Ser Met
        355                 360                 365

GAT ACC CGC TTC AAA CGC ATT ACC CTG CGT TTG GAC AGC CAT CCG TTG  1152
Asp Thr Arg Phe Lys Arg Ile Thr Leu Arg Leu Asp Ser His Pro Leu
    370                 375                 380
```

FIG. 8E

```
CAA CTC GGG GGG GGG CGA CAC CGC CTG TCG TTT AAA ACT TTC GCC AGC      1200
Gln Leu Gly Gly Gly Arg His Arg Leu Ser Phe Lys Thr Phe Ala Ser
385                 390                 395                 400

CGC CGT GAT TTT GAA AAC CTA AAC CGC GAC GAT TAT TAC TTC AGC GGC      1248
Arg Arg Asp Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Gly
                405                 410                 415

CGT GTT GTT CGA ACC ACC AGC AGT ATC CAG CAT CCG GTG AAA ACC ACC      1296
Arg Val Val Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr
            420                 425                 430

AAC TAC GGT TTC TCA CTG TCT GAC CAA ATT CAA TGG AAC GAC GTG TTC      1344
Asn Tyr Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe
        435                 440                 445

AGT AGC CGC GCA GGT ATC CGT TAC GAT CAT ACC AAA ATG ACG CCT CAG      1392
Ser Ser Arg Ala Gly Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln
    450                 455                 460

GAA TTG AAT GCC GAG TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC      1440
Glu Leu Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala
465                 470                 475                 480
```

FIG. 8F

```
AAC ACT TAT AAA GGC TGG AGC GGT TTT GTC GGC TTG GCG GCG CAA CTG      1488
Asn Thr Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu
                485                 490                 495

AAT CAG GCT TGG CGT GTC GGT TAC GAC ATT ACT TCC GGC TAC CGT GTC      1536
Asn Gln Ala Trp Arg Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val
            500                 505                 510

CCC AAT GCG TCC GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGT AAT      1584
Pro Asn Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn
        515                 520                 525

TGG CTG CCC AAT CCC AAC CTG AAA GCC GAG CGC ACG ACC ACC CAC ACC      1632
Trp Leu Pro Asn Pro Asn Leu Lys Ala Glu Arg Thr Thr Thr His Thr
    530                 535                 540

CTC TCT CTG CAA GGC CGC AGC GAA AAA GGT ACT TTG GAT GCC AAC CTG      1680
Leu Ser Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu
545                 550                 555                 560

TAT CAA AGC AAT TAC CGC AAT TTC CTG TCT GAA GAG CAG AAG CTG ACC      1728
Tyr Gln Ser Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr
                565                 570                 575
```

FIG. 8G

```
ACC AGC GGC GAT GTC AGC TGT ACT CAG ATG AAT TAC TAC TAC GGT ATG      1776
Thr Ser Gly Asp Val Ser Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met
            580                 585                 590

TGT AGC AAT CCT TAT TCC GAA AAA CTG GAA TGG CAG ATG CAA AAT ATC      1824
Cys Ser Asn Pro Tyr Ser Glu Lys Leu Glu Trp Gln Met Gln Asn Ile
        595                 600                 605

GAC AAG GCC AGA ATC CGC GGT ATC GAG CTG ACG GGC CGT CTG AAT GTG      1872
Asp Lys Ala Arg Ile Arg Gly Ile Glu Leu Thr Gly Arg Leu Asn Val
    610                 615                 620

GAC AAA GTA GCG TCT TTT GTT CCT GAG GGC TGG AAA CTG TTC GGC TCG      1920
Asp Lys Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser
625                 630                 635                 640

CTG GGT TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TCC      1968
Leu Gly Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser
                645                 650                 655

ACC CAG CCG TTG AAA GTG ATT GCC GGT ATC GAC TAT GAA AGT CCG AGC      2016
Thr Gln Pro Leu Lys Val Ile Ala Gly Ile Asp Tyr Glu Ser Pro Ser
            660                 665                 670
```

FIG. 8H

```
GAA AAA TGG GGC GTG TTC TCC CGC CTG ACC TAT CTG GGC GCG AAA AAG      2064
Glu Lys Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys
        675                 680                 685

GTC AAA GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC TGG GGT ACG      2112
Val Lys Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Trp Gly Thr
    690                 695                 700

CCT TTG CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT      2160
Pro Leu Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala
705                 710                 715                 720

TAT GTG TTC GAT ATG TAC GGC TTC TAC AAA CCG GTG AAA AAC CTG ACT      2208
Tyr Val Phe Asp Met Tyr Gly Phe Tyr Lys Pro Val Lys Asn Leu Thr
                725                 730                 735

TTG CGT GCA GGC GTA TAT AAT GTG TTC AAC CGC AAA TAC ACC ACT TGG      2256
Leu Arg Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp
            740                 745                 750

GAT TCC CTG CGC GGC CTG TAT AGC TAC AGC ACC ACC AAC TCG GTC GAC      2304
Asp Ser Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ser Val Asp
        755                 760                 765
```

FIG. 8I

```
CGC GAT GGC AAA GGC TTA GAC CGC TAC CGC GCC CCA AGC CGT AAT TAC      2352
Arg Asp Gly Lys Gly Leu Asp Arg Tyr Arg Ala Pro Ser Arg Asn Tyr
    770                 775                 780

GCC GTA TCG CTG GAA TGG AAG TTT TAA                                 2379
Ala Val Ser Leu Glu Trp Lys Phe  *
785                 790
```

FIG. 9A

```
ATG AAA CCA TTA CAC ATG CTT CCT ATT GCC GCG CTG GTC GGC AGT ATT      48
Met Lys Pro Leu His Met Leu Pro Ile Ala Ala Leu Val Gly Ser Ile
 1               5                  10                  15

TTC GGC AAT CCG GTC TTG GCA GCG GAT GAA GCT GCA ACC GAA ACC ACA      96
Phe Gly Asn Pro Val Leu Ala Ala Asp Glu Ala Ala Thr Glu Thr Thr
            20                  25                  30

CCC GTT AAA GCA GAG ATA AAA GAA GTG CGC GTT AAA GAC CAG CTT AAT     144
Pro Val Lys Ala Glu Ile Lys Glu Val Arg Val Lys Asp Gln Leu Asn
        35                  40                  45

GCG CCT GCA ACC GTG GAA CGT GTC AAC CTC GGC CGC ATT CAA CAG GAA     192
Ala Pro Ala Thr Val Glu Arg Val Asn Leu Gly Arg Ile Gln Gln Glu
    50                  55                  60

ATG ATA CGC GAC AAC AAA GAC TTG GTG CGT TAC TCC ACC GAC GTC GGC     240
Met Ile Arg Asp Asn Lys Asp Leu Val Arg Tyr Ser Thr Asp Val Gly
65                  70                  75                  80

TTG AGC GAT AGC GGC CGC CAT CAA AAA GGC TTT GCT GTG CGC GGC GTG     288
Leu Ser Asp Ser Gly Arg His Gln Lys Gly Phe Ala Val Arg Gly Val
                85                  90                  95
```

FIG. 9B


```
GAA GGC AAC CGT GTC GGT GTC AGC ATT GAC GGC GTG AGC CTG CCT GAT      336
Glu Gly Asn Arg Val Gly Val Ser Ile Asp Gly Val Ser Leu Pro Asp
               100                 105                 110

TCG GAA GAA AAC TCA CTG TAT GCA CGT TAT GGC AAC TTC AAC AGC TCG      384
Ser Glu Glu Asn Ser Leu Tyr Ala Arg Tyr Gly Asn Phe Asn Ser Ser
        115                 120                 125

CGC CTG TCT ATC GkC CCC GAA CTC GTG CGC AAC ATC GAA ATC GCG AAG      432
Arg Leu Ser Ile Asp Pro Glu Leu Val Arg Asn Ile Glu Ile Ala Lys
    130                 135                 140

GGC GCT GAC TCT TTC AAT ACC GGT AGC GGC GCA TTG GGT GGC GGC GTG      480
Gly Ala Asp Ser Phe Asn Thr Gly Ser Gly Ala Leu Gly Gly Gly Val
145                 150                 155                 160

AAT TAC CAA ACC CTG CAA GGA CAT GAT TTG CTG TTG GAC GAC AGG CAA      528
Asn Tyr Gln Thr Leu Gln Gly His Asp Leu Leu Leu Asp Asp Arg Gln
                165                 170                 175

TTC GGC GTG ATG ATG AAA AAC GGT TAC AGC AGC CGC AAC CGC GAA TGG      576
Phe Gly Val Met Met Lys Asn Gly Tyr Ser Ser Arg Asn Arg Glu Trp
               180                 185                 190
```

FIG. 9C

```
ACA AAT ACA CTC GGT TTC GGT GTG AGC AAC GAC CGC GTG GAT GCC GCT      624
Thr Asn Thr Leu Gly Phe Gly Val Ser Asn Asp Arg Val Asp Ala Ala
        195                 200                 205

TTG CTG TAT TCG CAA CGT CGC GGT CAT GAG ACC GAA AGC GCG GGC GAG      672
Leu Leu Tyr Ser Gln Arg Arg Gly His Glu Thr Glu Ser Ala Gly Glu
    210                 215                 220

CGT GGC TAT CCG GTA GAG GGT GCT GGC AGC GGA GCA ATT ATC CGT GGT      720
Arg Gly Tyr Pro Val Glu Gly Ala Gly Ser Gly Ala Ile Ile Arg Gly
225                 230                 235                 240

TCG TCA CGC GGT ATC CCT GAT CCG TCC AAA CAC AAA TAC CAC AAC TTC      768
Ser Ser Arg Gly Ile Pro Asp Pro Ser Lys His Lys Tyr His Asn Phe
                245                 250                 255

TTG GGT AAG ATT GCT TAT CAA ATC AAC GAC AAG CAC CGC ATC GGC CCA      816
Leu Gly Lys Ile Ala Tyr Gln Ile Asn Asp Lys His Arg Ile Gly Pro
            260                 265                 270

TCG TTT AAC GGC CAG CAG GGG CAT AAT TAC ACG ATT GAA GAG TCT TAT      864
Ser Phe Asn Gly Gln Gln Gly His Asn Tyr Thr Ile Glu Glu Ser Tyr
        275                 280                 285
```

FIG. 9D

```
AAC CTG ACC GCT TCT TCC TGG CGC GAA GCC GAT GAC GTA AAC AGA CGG      912
Asn Leu Thr Ala Ser Ser Trp Arg Glu Ala Asp Asp Val Asn Arg Arg
    290                 295                 300

CGC AAT GCC AAC CTC TTT TAC GAA TGG ACG CCT GAT TCA AAT TGG CTG      960
Arg Asn Ala Asn Leu Phe Tyr Glu Trp Thr Pro Asp Ser Asn Trp Leu
305                 310                 315                 320

TCG TCT TTG AAG GCG GAC TTC GAT TAT CAG ACA ACC AAA GTG GCG GCG     1008
Ser Ser Leu Lys Ala Asp Phe Asp Tyr Gln Thr Thr Lys Val Ala Ala
                325                 330                 335

GTT AAC AAC AAA GGC TCG TTC CCG ACG GAT TAT TCC ACC TGG ACG CGC     1056
Val Asn Asn Lys Gly Ser Phe Pro Thr Asp Tyr Ser Thr Trp Thr Arg
            340                 345                 350

AAC TAT AAT CAG AAG GAT TTG GAG AAT ATA TAC AAC CGC AGC ATG GAC     1104
Asn Tyr Asn Gln Lys Asp Leu Glu Asn Ile Tyr Asn Arg Ser Met Asp
        355                 360                 365

ACC CGA TTC AAA CGT TTT ACT TTG CGT ATG GAC AGC CAA CCG TTG CAA     1152
Thr Arg Phe Lys Arg Phe Thr Leu Arg Met Asp Ser Gln Pro Leu Gln
    370                 375                 380
```

FIG. 9E

```
CTG GGC GGC CAA CAT CGC TTG TCG CTT AAA ACT TTC GCC AGT CGG CGT      1200
Leu Gly Gly Gln Hls Arg Leu Ser Leu Lys Thr Phe Ala Ser Arg Arg
385                 390                 395                 400

GAG TTT GAA AAC TTA AAC CGC GAC GAT TAT TAC TTC AGC GAA AGA GTA      1248
Glu Phe Glu Asn Leu Asn Arg Asp Asp Tyr Tyr Phe Ser Glu Arg Val
                405                 410                 415

TCC CGT ACT ACC AGC TCG ATT CAA CAC CCC GTG AAA ACC ACT AAT TAT      1296
Ser Arg Thr Thr Ser Ser Ile Gln His Pro Val Lys Thr Thr Asn Tyr
            420                 425                 430

GGT TTC TCA CTG TCT GAT CAA ATC CAA TGG AAC GAC GTG TTC AGC AGC      1344
Gly Phe Ser Leu Ser Asp Gln Ile Gln Trp Asn Asp Val Phe Ser Ser
        435                 440                 445

CGT GCA GAT ATC CGT TAC GAT CAT ACC AAA ATG ACG CCT CAG GAA TTG      1392
Arg Ala Asp Ile Arg Tyr Asp His Thr Lys Met Thr Pro Gln Glu Leu
    450                 455                 460

AAT GCC GAG TGT CAT GCT TGT GAC AAA ACA CCG CCT GCA GCC AAT ACT      1440
Asn Ala Glu Cys His Ala Cys Asp Lys Thr Pro Pro Ala Ala Asn Thr
465                 470                 475                 480
```

FIG. 9F

```
TAT AAA GGC TGG AGC GGA TTT GTC GGT TTG GCG GCG CAA CTG AAT CAG      1488
Tyr Lys Gly Trp Ser Gly Phe Val Gly Leu Ala Ala Gln Leu Asn Gln
                485                 490                 495

GCT TGG CAT GTC GGT TAC GAC ATT ACT TCC GGC TAC CGT GTC CCC AAT      1536
Ala Trp His Val Gly Tyr Asp Ile Thr Ser Gly Tyr Arg Val Pro Asn
            500                 505                 510

GCG TCC GAA GTG TAT TTC ACT TAC AAC CAC GGT TCG GGT AAT TGG CTG      1584
Ala Ser Glu Val Tyr Phe Thr Tyr Asn His Gly Ser Gly Asn Trp Leu
        515                 520                 525

CCC AAT CCC AAC CTG AAA GCC GAG CGC AGC ACC ACC CAC ACC CTG TCT      1632
Pro Asn Pro Asn Leu Lys Ala Glu Arg Ser Thr Thr His Thr Leu Ser
    530                 535                 540

CTG CAA GGC CGC AGC GAA AAA GGT ACT TTG GAT GCC AAC CTG TAT CAA      1680
Leu Gln Gly Arg Ser Glu Lys Gly Thr Leu Asp Ala Asn Leu Tyr Gln
545                 550                 555                 560

AAC AAT TAC CGC AAC TTC TTG TCT GAA GAG CAG AAG CTG ACC ACC AGC      1728
Asn Asn Tyr Arg Asn Phe Leu Ser Glu Glu Gln Lys Leu Thr Thr Ser
                565                 570                 575
```

FIG. 9G


```
GGC GAT GTC GGC TGT ACT CAG ATG AAT TAC TAC TAC GGT ATG TGT AGC      1776
Gly Asp Val Gly Cys Thr Gln Met Asn Tyr Tyr Tyr Gly Met Cys Ser
            580                 585                 590

AAT CCT TAT TCC GAA AAA CCG GAA TGG CAG ATG CAA AAT ATC GAT AAG      1824
Asn Pro Tyr Ser Glu Lys Pro Glu Trp Gln Met Gln Asn Ile Asp Lys
        595                 600                 605

GCC CGA ATC CGT GGT CTT GAG CTG ACA GGC CGT CTG AAT GTG ACA AAA      1872
Ala Arg Ile Arg Gly Leu Glu Leu Thr Gly Arg Leu Asn Val Thr Lys
    610                 615                 620

GTA GCG TCT TTT GTT CCT GAG GGC TGG AAA TTG TTC GGC TCG CTG GGT      1920
Val Ala Ser Phe Val Pro Glu Gly Trp Lys Leu Phe Gly Ser Leu Gly
625                 630                 635                 640

TAT GCG AAA AGC AAA CTG TCG GGC GAC AAC AGC CTG CTG TCC ACA CAG      1968
Tyr Ala Lys Ser Lys Leu Ser Gly Asp Asn Ser Leu Leu Ser Thr Gln
            645                 650                 655

CCG CCG AAA GTG ATT GCC GGT GTC GAC TAC GAA AGC CCG AGC GAA AAA      2016
Pro Pro Lys Val Ile Ala Gly Val Asp Tyr Glu Ser Pro Ser Glu Lys
            660                 665                 670
```

FIG. 9H

```
TGG GGT GTG TTC TCC CGC CTG ACT TAT CTG GGT GCG AAA AAG GCC AAA      2064
Trp Gly Val Phe Ser Arg Leu Thr Tyr Leu Gly Ala Lys Lys Ala Lys
        675                 680                 685

GAC GCG CAA TAC ACC GTT TAT GAA AAC AAG GGC CGG GGT ACG CCT TTG      2112
Asp Ala Gln Tyr Thr Val Tyr Glu Asn Lys Gly Arg Gly Thr Pro Leu
    690                 695                 700

CAG AAA AAG GTA AAA GAT TAC CCG TGG CTG AAC AAG TCG GCT TAT GTG      2160
Gln Lys Lys Val Lys Asp Tyr Pro Trp Leu Asn Lys Ser Ala Tyr Val
705                 710                 715                 720

TTT GAT ATG TAC GGC TTC TAC AAA CTG GCT AAA AAC CTG ACT TTG CGT      2208
Phe Asp Met Tyr Gly Phe Tyr Lys Leu Ala Lys Asn Leu Thr Leu Arg
                725                 730                 735

GCA GGC GTA TAT AAT GTG TTC AAC CGC AAA TAC ACC ACT TGG GAT TCC      2256
Ala Gly Val Tyr Asn Val Phe Asn Arg Lys Tyr Thr Thr Trp Asp Ser
            740                 745                 750

CTG CGC GGT TTG TAT AGC TAC AGC ACC ACC AAC GCG GTC GAC CGA GAT      2304
Leu Arg Gly Leu Tyr Ser Tyr Ser Thr Thr Asn Ala Val Asp Arg Asp
        755                 760                 765
```

FIG. 9I


```
GGC AAA GGC TTA GAC CGC TAC CGC GCC TCA GGC CGT AAT TAC GCC GTA      2352
Gly Lys Gly Leu Asp Arg Tyr Arg Ala Ser Gly Arg Asn Tyr Ala Val
    770                 775                 780

TCG CTG GAT TGG AAG TTT TGA ATTCC                                  2378
Ser Leu Asp Trp Lys Phe  *
785                 790
```

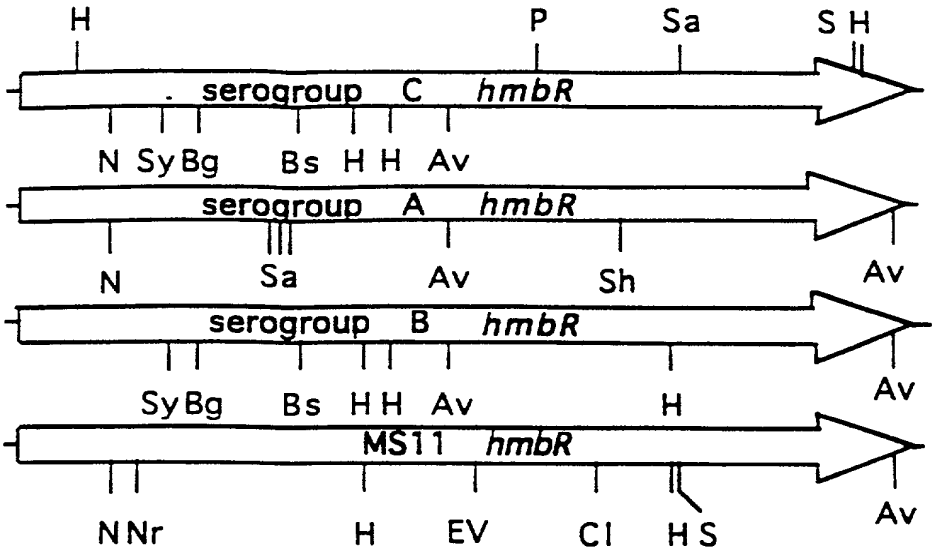

Figure 10

SHEET 43/47

FIG. 11A


```
HMBRA          MKPLQMLPIAALVGSIFGNPVLAADEAATETTPVKAEIKAVRVKGQRNAP   50
HMBRB          MKPLQMPPIAALLGSIFGNPVFAXDEAATETTPVKAEVKAVRVKGQRNAP   50
HMBRC          MKPLQMLPIAALVGSIFGNPVFAADEAATETTPVKAEVKAVRVKGQRNAP   50
HMBRMS11       MKPLHMLPIAALVGSIFGNPVLAADEAATETTPVKAEIKEVRVKDQLNAP   50
               ****.* *****.********.* **********.*.****.* ***

HMBRA          AAVERVNLNRIKQEMIRDNKDLVRYSTDVGLSDSGRHQKGFAVRGVEGNR  100
HMBRB          AAVERVNLNRIKQEMIRDNKDLVRYSTDVGLSDRSRHQKGFAIRGVEGDR  100
HMBRC          AAVERVNLNRIKQEMIRDNKDLVRYSTDVGLSDSGRHQKGFAVRGVEGNR  100
HMBRMS11       ATVERVNLGRIQQEMIRDNKDLVRYSTDVGLSDSGRHQKGFAVRGVEGNR  100
               *.******.**.**********************..*******.*****.*

HMBRA          VGVSIDGVNLPDSEENSLYARYGNFNSSRLSIDPELVRNIEIVKGADSFN  150
HMBRB          VGVSIDGVNLPDSEENSLYARYGNFNSSRLSIDPELVRNIDIVKGADSFN  150
HMBRC          VGVSIDGVNLPDSEENSLYARYGNFNSSRLSIDPELVRNIDIVKGADSFN  150
HMBRMS11       VGVSIDGVSLPDSEENSLYARYGNFNSSRLSIDPELVRNIEIAKGADSFN  150
               ********.*********************************.*.*******

HMBRA          TGSGALGGGVNYQTLQGRDLLLDDRQFGVMMKNGYSTRNREWTNTLGFGV  200
HMBRB          TGSGALGGGVNYQTLQGRDLLLPERQFGVMMKNGYSTRNREWTNTLGFGV  200
HMBRC          TGSGALGGGVNYQTLQGRDLLLPERQFGVMMKNGYSTRNREWTNTLGFGV  200
HMBRMS11       TGSGALGGGVNYQTLQGHDLLLDDRQFGVMMKNGYSSRNREWTNTLGFGV  200
               ******************.****  .************.*************
```

FIG. 11B


```
HMBRA       SNDRVDAALLYSQRRGHETESAGNRGYPVEGAGKETNIRGSARGIPDPSK   250
HMBRB       SNDRVDAALLYSQRRGHETESAGKRGYPVEGAGSGANIRGSARGIPDPSQ   250
HMBRC       SNDRVDAALLYSQRRGHETESAGKRGYPVEGAGSGANIRGSARGIPDPSQ   250
HMBRMS11    SNDRVDAALLYSQRRGHETESAGERGYPVEGAGSGAIIRGSSRGIPDPSK   250
            ************************.*********... ****.*******.

HMBRA       HKYHNFLGKIAYQINDNHRIGASLNGQQGHNYTVEESYNLTASSWREADD   300
HMBRB       HKYHSFLGKIAYQINDNHRIGASLNGQQGHNYTVEESYNLLASYWREADD   300
HMBRC       HKYHSFLGKIAYQINDNHRIGASLNGQQGHNYTVEESYNLLASYWREADD   300
HMBRMS11    HKYHNFLGKIAYQINDKHRIGPSFNGQQGHNYTIEESYNLTASSWREADD   300
            ****.***********.****.*.*********.****** ** ******

HMBRA       VNRRRNANLFYEWMPDSNWLSSLKADFDYQKTKVAAIN-KGSFPT-NYTT   348
HMBRB       VNRRRNTNLFYEWTPESDRLSMVKADVDYQKTKVSAVNYKGSFPT-NYTT   349
HMBRC       VNRRRNTNLFYEWTPESDRLSMVKADVDYQKTKVSAVNYKGSFPIEDSST   350
HMBRMS11    VNRRRNANLFYEWTPDSNWLSSLKADFDYQTTKVAAVNNKGSFPTD-YST   349
            ******.****** *.*..** .*** ***.***.*.* *****.    .*

HMBRA       WETEYHKKEVGEIYNRSMDTRFKRFTLRLDSHPLQLGGGRHRLSFKTFAS   398
HMBRB       WETEYHKKEVGEIYNRSMDTTFKRITLRMDSHPLQLGGGRHRLSFKTFAG   399
HMBRC       LTRNYNQKDLDEIYNRSMDTRFKRITLRLDSHPLQLGGGRHRLSFKTFAS   400
HMBRMS11    WTRNYNQKDLENIYNRSMDTRFKRFTLRMDSQPLQLGG-RHRLSLKTFAS   398
             . .*..*....******** ***.***.**.****** .****.****.
```

FIG. 11C

```
HMBRA       RRDFENLNRDDYYFSGRVVRTTSSIQHPVKTTNYGFSLSDQIQWNDVFSS  448
HMBRB       QRDFENLNRDDYYFSGRVVRTTNSIQHPVKTTNYGFSLSDQIQWNDVFSS  449
HMBRC       RRDFENLNRDDYYFSGRVVRTTSSIQHPVKTTNYGFSLSDQIQWNDVFSS  450
HMBRMS11    RREFENLNRDDYYFSERVSRTTSSIQHPVKTTNYGFSLSDQIQWNDVFSS  448
            .*.************.** ***.**************************

HMBRA       RAGIRYDHTKMTPQELNAECHACDKTPPAANTYKGWSGFVGLAAQLNQAW  498
HMBRB       RAGIRYDHTKMTPQELNADCHACDKTPPAANTYKGWSGFVGLAAQLSQTW  499
HMBRC       RAGIRYDHTKMTPQELNAECHACDKTPPAANTYKGWSGFVGLAAQLNQAW  500
HMBRMS11    RADIRYDHTKMTPQELNADCHACDKTPPAANTYKGWSGFVGLAAQLNQAW  498
            **.***************.****************************.*.*

HMBRA       RVGYDITSGYRVPNASEVYFTYNHGSGNWLPNPNLKAERSTTHTLSLQGR  548
HMBRB       RVGYDVTSGFRVPNASEVYFTYNHGSGTWKPNPNLKAERSTTHTLSLQGR  549
HMBRC       RVGYDITSGYRVPNASEVYFTYNHGSGNWLPNPNLKAERTTTHTLSLQGR  550
HMBRMS11    HVGYDITSGYRVPNASEVYFTYNHGSGNWLPNPNLKAERSTTHTLSLQGR  548
            ..***.***.******************.* *********.**********

HMBRA       SEKGMLDANLYQSNYRNFLSEEQKLTTSGTPGCTEENAYYSICSDPYKEK  598
HMBRB       GDKGTLDANLYQSNYRNFLSEEQNLTVSGTPGCTEEDAYYYRCSDPYKEK  599
HMBRC       SEKGTLDANLYQSNYRNFLSEEQKLTTSGDVSCTQMNYYYGMCSNPYSEK  600
HMBRMS11    SEKGTLDANLYQNNYRNFLSEEQNLTTSGDVGCTQMNYYYGMCSNPYSEK  598
            ..** *******.**********.**.**. .**. . **  **.**.**
```

FIG. 11D

```
HMBRA       LDWQMKNIDKARIRGIELTGRLNVDKVASFVPEGWKLFGSLGYAKSKLSG  648
HMBRB       LDWQMKNIDKARIRGIELTGRLNVDKVASFVPEGWKLFGSLGYAKSKLSG  649
HMBRC       LDWQMQNIDKARIRGLELTGRLNVDKVASFVPEGWKLFGSLGYAKSKLSG  650
HMBRMS11    PEWQMQNIDKARIRGLELTGRLNVTKVASFVPEGWKLFGSLGYAKSKLSG  648
             .***.*********.********.************************

HMBRA       DNSLLSTQPLKVIAGIDYESPSEKWGVFSRLTYLGAKKVKDAQYTVYENK  698
HMBRB       DNSLLSTQPLKVIAGIDYESPSEKWGVFSRLTYLGAKKVKDAQYTVYENK  699
HMBRC       DNSLLSTQPLKVIAGIDYESPSEKWGVFSRLTYLGAKKVKDAQYTVYENK  700
HMBRMS11    DNSLLSTQPPKVIAGVDYESPSEKWGVFSRLTYLGAKKAKDAQYTVYENK  698
            ********* *****.**********************.***********

HMBRA       GWGTPLQKKVKDYPWLNKSAYVFDMYGFYKPVKNLTLRAGVYNLFNRKYT  748
HMBRB       GWGTPLQKKVKDYPWLNKSAYVFDMYGFYKPAKNLTLRAGVYNLFNRKYT  749
HMBRC       GWGTPLQKKVKDYPWLNKSAYVFDMYGFYKPVKNLTLRAGVYNVFNRKYT  750
HMBRMS11    GRGTPLQKKVKDYPWLNKSAYVFDMYGFYKLAKNLTLRAGVYNVFNRKYT  748
            *.****************************  .***********.******

HMBRA       TWDSLRGLYSYSTTNAVDRDGKGLDRYRAPGRNYAVSLEWKF          790
HMBRB       TWDSLRGLYSYSTTNAVDRDGKGLDRYRAPGRNYAVSLEWKF          791
HMBRC       TWDSLRGLYSYSTTNSVDRDGKGLDRYRAPSRNYAVSLEWKF          792
HMBRMS11    TWDSLRGLYSYSTTNAVDRDGKGLDRYRASGRNYAVSLDWKF          790
            ***************.*************..*******.***
```

DECLARATION AND POWER OF ATTORNEY
(Case No. 94,784-A)

As below-named inventors, we hereby declare that:

Our residences, post office addresses and citizenship are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL BACTERIAL HEMOGLOBIN RECEPTOR GENES AND USES

which specification was filed on October 2, 1995 and given Serial No. 08/537,361

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

We further acknowledge the duty to disclose such material information which occurred between the filing date of the prior application, U.S. Serial No. 08/326,670, filed October 18, 1994 and the filing of this continuation-in-part application.

The undersigned hereby appoint the following:

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as our Attorneys and

Mark Chao Reg. No. 37293 Emily Miao Reg. No. 35285

as our Registered Patent Agents

the mailing address and telephone number of each of whom is BANNER & ALLEGRETTI, LTD., Ten South Wacker Drive, Chicago, Illinois 60606, and (312) 715-1000, with full power of substitution and revocation to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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